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<p>(54) Title: COMPLEMENTARILY BONDED TWO- AND THREE-DIMENSIONAL SUPRAMOLECULAR STRUCTURES</p> <p>(57) Abstract</p> <p>The present invention relates to supramolecules which are formed by at least two components. Each component comprises an effector molecule and at least one nucleic acid chain. The nucleic acid chains of each component are complementary to nucleic acid chains on other components and thus are able to bind the components of the supramolecule by the formation of double stranded nucleic acid chains between the complementary chains. The present invention also relates to a method of making the supramolecules of the present invention. The nucleic acid chains are preferably DNA, RNA and may also contain structural analogues of DNA or RNA. Effector molecules that may be used to form the supramolecules include, but are not limited to polypeptides, lipids, sugars. These effector molecules may impart chemical, physical properties to the supramolecule that include, but are not limited to hydrophobicity, hydrophilicity, electron conductivity, fluorescence, radioactivity, biological activity, cellular toxicity, catalytic activity, molecular and cellular recognition and <i>in vivo</i> transport selectivity.</p>		

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COMPLEMENTARILY BONDED TWO AND THREE DIMENSIONAL
SUPRAMOLECULAR STRUCTURES

1. FIELD OF THE INVENTION

5 The present invention is in the field supramolecular assemblies. More specifically, the present invention relates to supramolecular assemblies in which macromolecular components are bound together by polynucleotides.

10 2. BACKGROUND

Organized molecular systems are well known in biology and chemistry. For example, pure molecular compounds form crystals, and surface active molecular compounds form monolayers at air-water interphase and vesicles in water. 15 Bilayers of liposomes mimic biological membranes, and biological membranes are good examples of multimolecular organized systems. Viruses, in particular, are highly organized supramolecular assemblies whose complexity surpasses any man-made assembly. Another prime example is the DNA 20 double helix, which is the result of highly selective interaction of two complementary single strand molecules. Man made, or artificial examples of supramolecular systems, include cryptates, i.e., inclusion complexes of macrocyclic receptor molecules, and interrupting two dimensional hydrogen 25 bonded network by a large capping molecule. In these state-of-the-art examples, the structure of all participating molecules are highly specific.

Jean-Marie Lehn has defined supramolecular chemistry as the chemistry beyond individual molecules, i.e., the chemistry 30 of the intermolecular bond. For about twenty years, starting from early seventies, the supramolecular chemistry was limited into crown ethers and cryptates. These are based on the interaction of electron pair and ion and possibly additional ion-ion interaction (J.-M. Lehn, Angew. Chem. Int. Ed. Engl. 35 29 (1990) 1304-1319).

Oligobipyridines form in the presence of suitable metal cations such as copper(II) double-stranded helicates.

Auxiliary groups can be attached into bipyridine units. If these groups are nucleotides they can serve as recognition sites for DNA (U. Koert, M.M. Harding and J.-M. Lehn, Nature (1990) 346:339).

- 5 Most previously described hydrogen bonded supramolecules are supramolecular polymers, i.e., periodic supramolecules composed of one or two repeating units. In principle the number of repeating units of polymeric supramolecules can be larger than two but until now nobody has used more than two
- 10 repeating units. Examples of this class of supramolecules includes the chain-like supramolecule formed by co-crystallization of 1:1 mixture of 2,4,6-triaminopyrimidine and a suitable barbituric acid derivative (J.-M. Lehn, M. Mascal, A. DeCian, J. Fisher, J. Chem. Soc. Chem. Commun. (1990) 479).
- 15 Polymeric supramolecules formed from a single unit can have very interesting structures. For example, a tubular supramolecule has been formed from a single cyclic peptide (M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee and N. Khazanovich, Nature (1993) 366:324-327). These polymeric
- 20 supramolecules are often simply crystals or mixed crystals in which hydrogen bonding plays a predominant role in structure maintenance. Even, if these supramolecules are stable in solution, their size is variable like that of a conventional polymer.
- 25 A step towards controlling supramolecular size and shape has been the use of capping molecules to interrupt the molecular association at the desired point (J. P. Mathias, C. T. Seto, J. A. Zerkowski and G. M. Whitesides in "Molecular Recognition: Chemical and Biochemical Problems II" (Ed. S. M. Roberts) Royal Society of Chemistry). A mixture of the
- 30 isocyanurate derivative (benzCA₂) and trismelamine derivative (trisM₃) gives the supramolecule (trisM₃)₂(benzCA₂)₃. This strategy typically produces supramolecules which have 'molecular weight' of 4-10 KDa.
- 35 No process exists today for creating large molecular assemblies of deliberately chosen molecules in which the location of the molecules in the assembly can be selected

accurately with respect to each other. Nonetheless, a dire need exists for such molecular structures since they could have numerous important medical, chemical and physical applications. These applications include, but are not limited to, supramolecular drugs, drug delivery to target organs, capture of viruses and catalysts, sensors and nanotechnological components.

A large number of conjugates of oligonucleotides has been synthesized. These conjugates have been designed to be used as gene selective drugs and synthetic restriction enzymes. Other derivatives include oligonucleotides containing fluorescent or radioactive labels.

Polypeptides and proteins, especially enzymes, have been attached to oligonucleotides. A peptide or protein has been used as a tag for an oligonucleotide or oligonucleotide is used as a tag for a polypeptide. Techniques such as ELISA allowed to trace enzymes easier than oligonucleotides, enzymes were used as tags for oligonucleotides. PCR provides for assays of extreme sensitivity. Oligonucleotides are often used as a tag for polypeptides or peptidomimetics, so that the fate of the polypeptide can be followed *in vitro* or *in vivo*. Synthesis methods which are used to prepare these conjugates are also useful in this invention. (D. Pollard-Knight, Technique (1990) 3:113-132).

Linear single-stranded tRNA forms branched structures because there are several complementary pieces of the sequence are suitably located. Recently, several two and three dimensional structures have been formed using this principle (Y. Zhang and N. C. Seeman, J. Am. Chem. (1994) 116:1661-1669; N. C. Seeman, J. Theor. Biol. (1982) 99:237-247.). These DNA based supramolecules have been bound together to form active structures. Because several steps are typically needed to create these molecules, the overall synthesis yield can be very low (0.1-1 %) because of these steps alone.

Branched pre-mRNA is found in cells. These molecules have highly specific structures in which adenosine is always linked to guanosine. These branched RNAs have been

synthesised (T. Horn and M. S. Urdea Nucleic Acid. Res. (1989) 17:6959-6967; C. Sund, A. Földesi, S.-I. Yamakage and J. Chattopahyaya, Nucleic Acid. Res. (1991) 9-12). The synthesis of branched nucleic acids has been extended to the synthesis of nucleic acid dendrimers (R. H. E. Hudson and M. J. Damha, J. Am. Chem. Soc. (1993) 113:2119-2124).

Oligonucleotide comb and fork structures have been used for analytical purposes (M. S. Urdea, B. Warner, J. A. Running, J. A. Kolberg, J. M. Clyne, R. Sanchez-Pescador and T. Horn (Chiron Corp.) PCT Int. Appl. No. WO 89 03,891 (cl. C12Q1/68), 05 May 1989, U.S. Appl. No. 109,282, 15 October 1987. 112 pp).

All previously known supramolecular structures have some drawbacks. It is of interest to provide novel supramolecular structures that may be adapted for a variety of uses, including disease therapy, diagnostics, assays, and electronics.

3. SUMMARY OF THE INVENTION

The present invention relates to supramolecules which are formed by at least two component molecules. Each component molecule comprises at least one effector molecule and at least one nucleic acid chain. At least one of the nucleic acid chains on at least one component molecule of the supramolecules of the invention are complementary to nucleic acid chains on at least one other component, and thus are able to bind the components of the supramolecule by the formation of double stranded nucleic acid chains between the complementary chains. The present invention also provides methods of making the supramolecules of the present invention.

The nucleic acid chains are preferably DNA, RNA and may also contain structural analogues of DNA or RNA. Effector molecules that may be used to form the supramolecules include, but are not limited to polypeptides, proteins, lipids, sugars. These effector molecules may impart chemical and physical properties to the supramolecule include, hydrophobicity, hydrophilicity, electron conductivity, fluorescence,

radioactivity, biological activity, cellular toxicity, catalytic activity, molecular and cellular recognition and in vivo transport selectivity.

Another aspect of the invention is to provide
5 supramolecular structures of the invention that may be used to treat or prevent infectious diseases, particularly viral infectious diseases. Supramolecular structures suitable for the treatment and/or prevention of infectious diseases
comprise effector molecules that are antibodies specific for
10 one or more antigen on a viral particle and one or more enzyme capable of catalyzing a reaction that destroys the infectivity of the virus of interest, e.g., hydrolysis of viral coat proteins.

An effector molecule can also be a toxin, such as ricin,
15 which will kill the cell, if the virus is internalized. Another aspect of the invention is to provide supramolecular structures adapted for the treatment of non-infectious diseases. Supramolecular structure for the treatment of specific diseases may comprise effector molecules specific for
20 certain cells or tissues and effector molecules that serves to directly alleviate a given disease condition.

Another aspect of the invention is to provide supramolecular structures that expedite the delivery of polynucleotides and other macromolecules into the interior of
25 cells. Supramolecular structures of the invention adapted for the internalization of macromolecules may comprise effector molecules that either alone, or in combination with other effector molecules, on the same or different structure, that are capable of crosslinking receptors on the surface of a cell
30 for transformation.

Another aspect of the invention is to provide supramolecular constructions useful for performing assays for compounds of interest, particularly immunoassays. Supramolecular structures for use in assays typically comprise
35 an effector molecule capable of specifically binding to a compound of interest and a second effector molecule that may be capable of producing a detectable signal, e.g., an enzyme, or

a second molecule capable of specifically binding to a compound of interest. Another aspect of the invention is to provide assays employing supramolecular constructions of the invention.

5 Another aspect of the invention is to provide supramolecular constructions useful for the prevention and treatment of atherosclerosis and related cardiovascular disorders. Supramolecular structures of the invention useful for the treatment of such diseases may comprise an effector
10 molecule that is an antibody specific for antigens in atherosclerotic plaque.

4. BRIEF DESCRIPTION OF THE FIGURES

The invention will be better understood by reference to
15 the appended Figures, of which:

Figure 1 is a schematic representation of the construction of a supramolecule constructed from two components. Two effector molecules, M and N are connected by complementary nucleic acid strands. The effector molecules are
20 represented by circles. The two connected complementary nucleic acid strands are depicted by a rectangle.

Figure 2(A) is a schematic representation of the construction of a square planar supramolecule constructed from
25 four components.

Figure 2(B) is a schematic representation of the construction of a square planar supramolecule constructed from four components which is reinforced by diagonal double stranded nucleic acid chains.

30 Figure 2(C) is a schematic representation of the construction of a tetrahedral supramolecule constructed from four components.

Figure 3(A) is a schematic representation of the construction of an antibody-multienzyme supramolecule
35 constructed from supramolecular components.

Figure 3(B) is a schematic representation of the construction of supramolecular subcomponents used in Figure

3(A) from molecules each containing one enzyme or antibody.

Figure 3(C) is a schematic representation of the construction of two supramolecules containing an antibody and two enzymes. The combination of these two supramolecules is
5 able to degrade all lipid components of the virus.

Figure 4 is a schematic representation of a supramolecule subcomponent which is capable of forming a supramolecular cage around a virus when it combines with a complementary supramolecule subcomponent.

10 Figure 5 is a schematic representation of the construction of a supramolecule for surrounding an icosahedral virus. Figure 5A is a schematic representation of a typical icosahedral virus. Figure 5B is a schematic representation of the supramolecule subcomponent of Figure 4 approaching the
15 icosahedral virus. Figure 5C depicts a second, complementary supramolecule subcomponent approaching the icosahedral virus. Figure 5D depicts two complementary supramolecule subcomponents surrounding a icosahedral virus. Figure 5E depicts a icosahedral virus encased within a supramolecule.

20 Figure 6 is a schematic representation of how the analogous structure for the large molecule in Figure 4 can be prepared using smaller molecules.

Figure 7 is a schematic representation of molecules needed to construct the supramolecule of Figure 5.

25 Figure 8 is a schematic representation of supramolecular assemblies which give analogous structures to the two molecules shown in Figure 6.

Figure 9 is a schematic representation of the use of triple helices in supramolecular assemblies.

30 Figure 10 illustrates an example of a spacer molecule for connecting three nucleotides to an effector molecule.

Figure 11 illustrates an example of a second spacer molecule for connecting three nucleotides to an effector molecule.

35 Figure 12 illustrates an example of a method for cross-linking two complementary oligonucleotides at one end.

Figure 13 illustrates an example of the coupling of two

derivatized peptide chains to form a branched peptide structure which can serve as a trivalent linker.

Figure 14 illustrates an example of a method of using the manipulation of protective groups on a trivalent spacer in order to use the trivalent spacer in oligonucleotide synthesis.

Figure 15A is a schematic representation of a supramolecule adapted for transformation of a nucleic acid of interest into a eukaryotic cell. Figure 15B is a schematic representation of a supramolecule adapted for transformation into a cell. Figure 15C is a schematic representation of a supramolecule adapted for transforming a cell and the internalization, i.e., transformation process.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to supramolecules (also referred to herein as supramolecular assemblies and supramolecular constructions) that comprise at least two components, i.e., supramolecular components. Each supramolecular component comprises an effector molecule and at least one nucleic acid chain covalently joined to the effector molecule. By placing complementary nucleic acid chains on different components, the components of the supramolecule may be bound together by the associative forces, i.e., hydrogen bonding, between the complementary nucleic acid chains, thereby producing supramolecular constructions in which two or more effector molecules are joined to one another a double-stranded or partially double stranded nucleic acids.

The general concept of the present invention may be better understood by reference to Figure 1 wherein supramolecular components A and B are joined by effector molecules M and N, respectively. Components A and B are bound to each other by the double stranded nucleic acid chain formed by complementary nucleic acid chains.

There is no theoretical limit to the number of supramolecular components that may be used to construct a particular supramolecule. Rather, steric factors that could

limit the number of components that can be used in a particular supramolecule may be avoided by proper design of the supramolecule using basic structural information that is well known to the person of ordinary skill in the art of
5 biochemistry. Thus the invention provides for numerous compounds that are supramolecular assemblies, i.e., supramolecules, comprising two or more supramolecular components of the invention. The supramolecular components of the invention comprise an effector molecule, e.g., an
10 antibody, covalently joined to at least one polynucleotide. Two or more supramolecular components of the invention may be joined to one another by means of the nucleic acids moieties of the supramolecular components by employing nucleic acids that have regions of complementarily or partial
15 complementarily to one another. Thus two or more effector molecules may be joined to one another by double stranded or partially double stranded nucleic acids.

Any molecule can be used as effector molecule portion of the subject supramolecule and supramolecular components.
20 Suitable molecules for use as the effector molecule moieties of the supramolecular components of the invention include, but are not limited to, sugars, peptides, lipids, polymers. The effector molecules of the supramolecule may serve several different functions within the supramolecules. For example,
25 the effector molecules may be used to provide a wide array of structural features to the supramolecule. In addition, the effector molecules can also provide certain chemical and physical properties to the supramolecules which include, but are not limited to, hydrophobicity, hydrophilicity, electron
30 conductivity, fluorescence, radioactivity, biological activity, cellular toxicity, catalytic activity, as well as molecular and cellular recognition and *in vivo* transport selectivity. Effector molecules include a variety of protein type, including toxins, proteinases, receptors, ligands,
35 lectins, antibodies, esterases, hormones, cell surface markers, etc.

The nucleic acid used to join the subject supramolecular

components to each other are preferably between 5 and 100 bases in length, although nucleic acids may be significantly longer than 100 bases. The nucleic acid portion of the subject supramolecular components and supramolecular assemblies may be any of the wide variety nucleic acid molecules, either naturally occurring, e.g., RNA or DNA, or synthetic analogs, e.g., phosphorothioates. The term "nucleic acids" as used herein, unless indicated otherwise, refers to both naturally occurring nucleic acids and synthetic analogs thereof. For many applications, it may be desirable to use synthetic analogs of natural nucleic acid rather than nucleic acids because of certain properties specific to the analogs e.g., nuclease resistance and higher denaturation temperatures of double-stranded nucleic acids.

Detailed descriptions on the use and synthesis of nucleic acid analogs can be found, among other places, in U.S. Patent No. 5,292,875 (phosphorothioates), U.S. Patent No. 5,218,103 (thiophosphoramidites), U.S. Patent No. 5,183,885 (phosphorothioates), U.S. Patent No. 5,151,510 (phosphorothioates), U.S. Patent No. 4,814,448 (phosphonates), U.S. Patent No. 4,096,210 (phosphorates) U.S. Patent No. 4,094,873 (phenylphosphorothioates), Ragle et al., Nuc. Acids. Res. 18(6):4751-4757 (1990) (phosphoramidates). Information on how to synthesize conventional nucleic acid can be found, among other places, in Eckstein Oligonucleotide and Analogues: A Practical Approach Oxford University Press (1992). The complementary nucleic acids need not necessarily be entirely complementary with respect to one another. A nucleic acid of one of a first supramolecular component may be complementary to only a portion of the nucleic acid moiety of a second supramolecular component or the complementarity may be over the entire length of the nucleic acid. Nucleic acid moieties of the subject supramolecular components may contain multiple regions of complementarity to two or more nucleic acids moieties on additional supramolecular components thereby to be joined to permitting three or more supramolecular components to be joined to one another through hybridization. The

complementarily (as measured by sequence homology) may be either 100 percent or less. It will be appreciated by those of ordinary skill in the art that the strength of associating, as indicated by duplex nucleic acid melting point, may be modulated by controlling factors such as the degree of complementarily, the identity of the base pairs (e.g., GC rich nucleic acids have a higher T_m than AT rich nucleic acids), the choice of a nucleic acid or nucleic acid analog, the length of the region of complementarily, and the like. The nucleic acid moieties of the subject supramolecular components may be linear or branched. Methods of producing branched nucleic acids are known to the person skilled in the art, and example of how to make branched nucleic acid molecules can be found in PCT Publication No. WO 89/03891. The use of branched nucleic acids as the nucleic acids as the nucleic acid moieties of the subject supramolecular components is particular interest because branched nucleic acid may be used to conveniently join three or more supramolecules components to one another through hybridization of the nucleic moieties. Triple and tetra helixes of nucleic acid chains can also be used in the supramolecules in order to provide other structural characteristics, such as rigidity, to the supramolecule.

The length of the nucleic acid moieties as well as the position of the complementary base on the nucleic acids may be used to control the two and three dimensional shape of the supramolecule. For example, as depicted in Figure 2(A), a square supramolecule can be prepared by employing four components which each contain two nucleic acid chains of equal length. Similarly, as also depicted in Figure 2(C), a tetrahedral supramolecule can be formed using four components. As can be seen from Figures 2(A) and 2(C), a wide variety of two and three dimensional supramolecule structures may be formed using differing numbers of components and differing numbers of complementary nucleic acid chains. For example, supramolecules of the present invention may contain geometric configurations that generally resemble triangles, squares,

pentagons, hexagons, heptagons, octagons, parallelograms, pyramids, tetrahedrons, cubes and cylinders. It should also be understood that these figures are merely schematic representations of supramolecular assemblies and that the
5 supramolecule may not actually possess these geometric structures in solution or in crystalline form because of the due to the flexibility of double stranded nucleic acid chains as well as other solvation, electronic and stearic factors that may be present in a given supramolecule.

10 With respect to each supramolecular component, the number of nucleic acid moieties that may be attached to a particular effector molecule may be varied greatly so as to produce supramolecular assemblies of the desired structure. Supramolecular components of the invention may comprise one or
15 more nucleic acid moieties. The total number of nucleic acid moieties that may be attached to an effector molecule is limited by stearic hinderance and the number of potential attachment sites, problems which may be avoided by proper selection of the effector molecule and the nucleic acid
20 moieties.

In another embodiment of the supramolecular components of the invention, more than one effector molecules may be joined to a single nucleic acid molecule. Such supramolecular components comprising a plurality of effector molecules joined
25 to a single nucleic acid molecule may be used to form supramolecular assemblies through a nucleic acid hybridization with the nucleic acid moieties of similar supramolecular components or supramolecular components in which nucleic acid moieties are joined to only a single effector molecule.

30 The supramolecular assemblies of the invention may be produced in a variety of environments, either *in vitro* or *in vivo*. Supramolecular assemblies may be constructed *in vitro* by mixing two or more supramolecular components having complementary nucleic acids. Conditions in the *in vitro*
35 reaction mixture may be varied so as to influence the rate of supramolecular assembly formation and the nature of the supramolecular assemblies produced.

The supramolecules of the present invention may be used in a very wide variety of applications which include, but are not limited to treatment of infectious disease, including HIV-1 infections, treatment of atherosclerosis, treatment of
5 cancer, immunoassays, self-assembling resist materials, for electronic self-assembling nanocircuitry, catalytic clusters, sensors, supramolecular drugs, which are capable of caging, i.e., encapsulating viruses and/or destroying viruses. Drug and enzyme targeting to cells and viruses may be enormously
10 improved by using supramolecular assemblies of the invention comprising many similar or different monoclonal antibodies and several drug molecules, enzymes or other effector molecules.

It will be appreciated by the person of ordinary skill in the art that the therapeutic embodiments of the supramolecules
15 of the invention (e.g., supramolecules for the treatment of cancer, viral infections, atherosclerosis) also include supramolecules in which effector molecules are joined to one another through conventional, i.e., non-polynucleotide, linkers. The use of non-polynucleotide linkers is well known
20 the person of ordinary skill in the art and is described in, among other places, in several volumes of the series Methods in Enzymology, Academic Press, San Diego CA. Examples of such non-polynucleotide linkers include, 4-benzoylbenzoic Acid N-hydroxysuccinimide esters, 3-maleimidobenzoic acid N-
25 hydroxysuccinimide esters, 1,4-phenyleneisothiocyanates, and the like. In those embodiments of the invention in which non-polynucleotide linkers are used to join effector molecules, it may be advantageous to administer a mixture of different effector molecule conjugates to a patient rather than a large
30 supramolecule. In the treatment of HIV-1 infection for example, rather than administer a single supramolecule comprising (i) an anti-gp120 macromolecule, (ii) a phospholipase, and (iii) a proteinase, it may be desirable to administer a formulation comprising (i) an anti-gp120-
35 phospholipase conjugate and (ii) an anti-gp120-protease conjugate.

When the supramolecular assemblies and supramolecular

components of the invention are used *in vivo*, the compounds are typically administered in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the
5 therapeutic proteins and nucleic acids to the patient.

Sterile water, alcohol, fats, waxes, and inert solids may be included in the carrier. Pharmaceutically accepted adjuvants (buffering agents, dispersing agent) may also be incorporated into the pharmaceutical composition.

10 The supramolecular assemblies and supramolecular components of the invention may be administered to a patient in a variety of ways. Preferably, the pharmaceutical compositions may be administered parenterally, *i.e.*,
subcutaneously, intramuscularly or intravenously. Thus, this
15 invention provides compositions for parenteral administration which comprise a solution of the human monoclonal antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, water, buffered water, 0.4% saline, 0.3%
20 glycerine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate
25 physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, *e.g.*, from less than
30 about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable
35 compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's

Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pa (1980), which is incorporated herein by reference.

An important use supramolecules of the invention is a 2-dimensional supramolecular structures on semiconductor or
5 other electrically conductive surfaces so that desired patterns of self-assembling resist materials may be conveniently formed. Thus, the use of X-rays and electron beam lithography may be avoided when creating nanometerscale patterns on the semiconductor surfaces. This capability will
10 make a completely new order of nanoelectronics possible.

A second application of major importance will be self-assembling nanocircuitry using this technique. Preprepared diodes, transistors, capacitors, resistors, etc. and wires can be connected in highly selective ways to form two or three
15 dimensional electronic entities. Electronically conducting complementary polynucleotide chains may be used when electric contact of the nanocomponents is needed.

The supramolecular assemblies of the present invention may also be used in catalytic and sensor applications. For
20 example, tetrahedral or similarly shaped supramolecular assemblies may be used to create supramolecular assemblies of catalytic clusters comprising several enzymes. Employing the supramolecule methodology of the present invention, enzymes may be attached to a surface in an organized fashion in order
25 to create desired sequential reaction. With regard to sensor applications, a sensor may be created that contains additional biomolecules or organic molecules that give a photonic or electrical signal when a molecule of interest is the supermolecular assembly sensor.

30 The supramolecular constructions and supramolecular components of the invention may be used to provide novel immunoassays and related assays for the detection of compounds of interest. Immunoassay technology is highly developed and well known to person of ordinary skill in the art, see, for
35 example, Hudson, Practical Immunology 3rd Ed. Oxford Publication (1989), and Catty Antibodies: A Practical Approach Volumes 1 & 2 Oxford University press (1989). Conventional

immunoassays typically employ antibodies conjugated to enzymes, and/or antibody-antibody conjugates. It will be appreciated by one skilled in the art that many embodiments of the supramolecular assemblies of the invention may be substantiated for the conventional antibody conjugates used in conventional assays. Supramolecular constructions and supramolecular components of the invention useful or assay comprise at least one member of a specific binding pair (e.g., an antibody, where the specific binding pair of molecules is an antibody and antigen target) as an effector molecule portion of a supramolecular component. Such supramolecular components may be used form supramolecular constructions use of assays, such supramolecules may, for example comprise (1) two or more specific binding pair members, e.g., antibodies, (2) an antibody and an enzyme capable of generating a detectable signal, e.g., alkaline phosphatase.

Numerous advantageous variants of conventional immunoassays are enabled by employing the supramolecular assemblies of invention instead of conventional antibody conjugates because the supramolecular assemblies of the invention may be assembled, disassembled, or reassembled during an assay due to the ability of the double-stranded nucleic acid moieties of the assembly to disassociate or removal of the appropriate conditions. For example, (i) a supramolecular assembly comprising antibody joined by a double stranded nucleic acid molecule to an enzyme producing a detectable signal may be bound to a target antigen of interest, (ii) the supramolecular assembly may then be disassociated so as to release the supramolecular component comprising the enzyme effector molecule (iii) the bound supramolecular component may then be used to form a new supramolecular assembly with a new supramolecular comprising a second antibody as an effector molecule, thereby permitting the immobilization of a second molecule of interest at the same location as the bound supramolecular assembly. A person of ordinary skill in the art will appreciate that the properties the subject supramolecular structures permit many

new and useful assay procedures to be performed.

The supramolecular structures of the invention may be adapted so as to prevent or treat various infectious diseases, including HIV-1, the etiological agent of AIDS. Specific
5 infections organisms may be targeted by creating, and administering in an effective amount, supramolecular structure of the invention comprising as effector molecules, (1) an antibody specific for molecule on the infectious agent and (2) an enzyme capable of catalyzing the modification of some
10 integral structure of the infectious agent. For example, a supramolecular structure adapted for the control of HIV-1 may comprise an antibody specific for HIV-1 component, e.g., gp120, and one or more of the following enzymes (1) a phospholipase A₂, (2) a lipase, (3) a cholesterol esterase.
15 By including such enzymes in a supramolecular construction, the lipid bilayer coat the infectious viral particle that may be destroyed. Additionally, supramolecular structures of the invention adapted for the treatment of the infectious disease may further comprise of protease capable of degrading a
20 protein component of the infectious agent and/or a single stranded nucleic acid capable of hybridizing to a portion of the genome of the infectious organism of interest. In other embodiments of the supramolecules of the invention for the treatment/prevention of HIV-1 infections, soluble CD4 (e.g.,
25 TT4) may be used as effector molecule to provide viral target specificity.

The principles presented in this application enable the purposeful construction of huge molecular assemblies having an exactly known chemical structure. For example, in Example 6,
30 as shown in figures 5A-D, describes the construction of a supramolecule for capturing virus particles which would have a molecular weight of about 4,000,000 Daltons.

The present invention provides the particular advantage that the precise molecular weight and chemical structure of
35 the supramolecule is under the complete control of the chemist constructing the molecule. This is in sharp contrast to polymer chemical methods which allow only approximate control

of the mean molecular weight and branching.

Another aspect of the invention is to provide supramolecules adapted so as to mediate the transfer of polynucleotides of interest into a host cell, i.e.,
5 transfection or transformation. Supramolecules of the invention for cell transfection comprise effect of molecules capable of initiation the natural internalization machinery of a eukaryotic cell. Such effector molecules e.g., antibodies, are capable of binding to cell surface molecules, e.g.,
10 receptors, and preferably cross-linking the receptors when the effector molecules are components of a supramolecular assembly of the invention. A supramolecular assembly comprising multiple antibodies may increase chances of internalization by increasing the concentration of cross-linked cell-surface
15 molecules. Additionally, sets of supramolecular components of the invention may be used to transform cells by employing the internalization machinery of the cell. For example, a first supramolecular component consisting of a cell surface receptor specific antibody joined to a nucleic acid moiety and a second
20 supramolecular component consisting of a second cell surface receptor-specific antibody joined to a complementary nucleic acid moiety. By permitting the first and second supramolecular component nucleic acids to hybridize to one another after the antibody moieties have bound to a cell
25 surface, receptor cross-linking, and hence internalization, may be achieved. Supramolecular assemblies of the invention may also comprise additional nucleic acids for internalization into a host cell of interest. Nucleic acid components of supramolecular assemblies for cell transformation may be
30 detached from the supramolecular assembly in a variety of ways. As shown in Figure 15A, the nucleic acid may be detached through the use of restriction enzymes or other nucleases. Additionally, nucleic acid components may detach from supramolecular assemblies through the process of nucleic
35 acid denaturalization, provided the nucleic acids are not covalently attached to the assembly. In another embodiment of the subject Supramolecular assemblies for transformation,

effector molecules having phospholipase A₂ activity may be used to introduce pores into a cell membrane. In other embodiments of the invention, the supramolecular assembly may comprise polyamines, e.g., spermine so as to mediate transformation.

The large scale solid phase synthesis (e.g., over 1 mmole) of oligonucleotides is difficult to achieve using previously described synthesis methods. A significant problem with large scale synthesis is the efficient mixing of the heterogeneous system. Silica, polystyrene or other similar solid support particles (typically spherical) modified with polyethyleneoxide chains are commonly used as a support for oligonucleotide synthesis. The growing oligonucleotide chains may form coils and stacking relationships, even between oligonucleotides on separate support particles, thereby creating a network that can prevent the efficient entry of reagents. The higher density of these spherical particles also makes efficient reaction mixing even more difficult.

Large scale synthesis of oligonucleotide, e.g., 0.1-1 mole, is useful for the commercial scale production of supramolecules and supramolecular components of the invention. The following improvements of the current oligonucleotide synthesis procedure solve the above-described problems surrounding large scale synthesis of oligonucleotides. First, acetonitrile is replaced with a solvent or solvent mixture that has a specific density of about one and that is also better able to solvate the heterocyclic bases of nucleotides than acetonitrile. Suitable solvents having these desired properties include benzonitrile or a mixture of acetonitrile and dichlorobenzene. The density of these solvents is compatible with the use of polystyrene or comparable solid supports. Solid supports will float in these preferred solvents, thereby permitting mixing steps to be easily performed. Another improvement over conventional oligonucleotide synthesis that may be used to effect large scale synthesis is the exposure of the reaction mixture to microwaves during the coupling step. Microwaves increase

molecular rotation and reduce unwanted polynucleotide uncoiling and network formation without testing the reaction mixture to excessive heat. An additional improvement over conventional oligonucleotide methods synthesis is instead of 5 monomeric amidites, dimeric or trimeric amidites may be used as building blocks. Even larger amidite multimers may be used to construct oligonucleotides; however, monomeric, dimeric and trimeric amidites and their combinations are preferred. Using dimers and trimers as building blocks requires preparation of 10 16 dimer amidites and up to 64 trimer amidites separate. The use of multimeric amidites the number of couplings during automated synthesis is decreased significantly and accordingly the yield and purity is increased. The three above-described oligonucleotide improvements may be employed separately or in 15 combination with one another. A person of ordinary skill in the art will appreciate that an ideal combination of the above-described improvements will depend upon the length of the oligonucleotide described and the scale of the synthesis.

The invention having been described above may be better 20 understood by reference to the following examples. The following examples are offered in order to illustrate the invention and should not be interpreted as limiting the invention

25

EXAMPLES

1. Illustration of Complementary Nucleic Acid Sequences

Table 1 provides examples of nucleic acid sequences and their complementary sequences that may be used in the present invention; the construction of complementary nucleic acids is 30 known to the person of ordinary skill in the art.

For the purpose of these examples, complementary chains of nucleic acids are depicted as an integer and that integer underlined. For example, $-(A_n-C_p)_i$ is identified as 1 in Table 1. Its complement, $-(T_n-G_p)_i$ is labelled 1. With regard to 35 the indices n, p, q and r used in Table 1, it should be understood that these indices are independent for each set of complementary nucleic acid chains.

TABLE 1

	Chain	Unit	Complementary Unit	Complementary	Chain
		1 - (A _n -C _p) i	(T _n -G _p) i-		<u>1</u>
5		2 - (A _n -T _p) i	(T _n -A _p) i-	n≠p ^a	<u>2</u>
		3 - (C _n -G _p) i	(G _n -C _p) i-	n≠p ^a	<u>3</u>
		4 - (A _n -C _p -G _q) j	(T _n -G _p -C _q) j-		<u>4</u>
		5 - (A _n -G _p -C _q) j	(T _n -C _p -G _q) j-		<u>5</u>
10		6 - (A _n -C _p -T _q) j	(T _n -G _p -A _q) j-		<u>6</u>
		7 - (A _n -T _p -C _q) j	(T _n -A _p -G _q) j-		<u>7</u>
		8 - (A _n -C _p -A _q -G _r) j	(T _n -G _p -T _q -C _r) j-		<u>8</u>
		9 - (A _n -G _p -A _q -C _r) j	(T _n -C _p -T _q -G _r) j-		<u>9</u>

^a If n=p then the oligonucleotide is self-complementary and can be useful when similar units are coupled together.

Many of the examples given herein are provided in order to demonstrate the principles of the invention. Preferably, repeating units are avoided.

DNA and RNA triple helices are also well known and may be used to form the supramolecular assemblies of the invention. Triple helices may result from the association between T....A....T and C....G....C. As a result, nucleic acid changes, such as those listed in Table 2 can be used to form triple helices to bind different components of a supramolecule. One advantage in using triple helix structures is increased rigidity. This property can be utilized even after the supramolecule has been assembled. Triple helix forming oligonucleotides may be used as the nucleic acid moieties of the supramolecular components of the invention. Double helical structures, which are capable of binding to a third oligonucleotide, do so and give rigidity and shape to the supramolecule. The use of triple helices in supramolecular assemblies is demonstrated in Figure 8.

TABLE 2

	Center coil	Two outer coils
11	$-A_h$	T_h-
12	$-G_h$	C_h-
13	$-(A_n-G_p) i$	$(T_n-C_p) i-$
14	$-(A_n-A_p-G_q) j$	$(T_n-T_p-C_q) j-$
15	$-(A_n-G_p-G_q) j$	$(T_n-C_p-C_q) j-$

10 2. Construction Of A Square Planar Supramolecule

Figure 2(A) depicts the construction of a square planar supramolecule from four different components. Component A comprises effector molecule M to which is attached nucleic acid chains 1 and 2. Component B is formed by attaching nucleic acid chains 1 and 3 to effector molecule N. Component C is formed by attaching nucleic acid chains 2 and 3 to effector molecule P. Component D is formed by attaching two nucleic acid chains 3 to effector molecule Q. When components A, B and C are mixed, the complementary chains 2 and 2 of components A and C bind and the complementary chains 1 and 1 of components A and B bind. When component D is added, the 3 nucleic acid chains bind to the 3 chains of components A and C to form the square supramolecule depicted in Figure 2(A).

Figure 2(B) depicts how the square planar supramolecule can be stabilized by the addition of complementary nucleic acid chains that bind component A and C to component B to D. Since the distance between diagonally positioned effector molecules are 1.41 times the distance between effector molecules on the sides of the square supramolecule, the complementary nucleic acid chains used to bind the effector molecules diagonal to one another must be at least 1.41 times as long as the complementary nucleic acid chains binding the adjacent effector molecules in order to produce a supramolecular assembly with the desired shape.

35

3. Construct Of A Tetrahedral Supramolecule

Figure 2(C) depicts the construction of a tetrahedral supramolecule using four components. In order to form a tetrahedral supramolecule component A is attached to
5 components B, C and D by complementary nucleic acid chains. Similarly, components B, C and D are attached to the components by complementary nucleic acid chains.

4. Synthesis Of Components Of Supramolecules

10 A. Preparation of Nucleic Acid Chains

Several different high yield strategies for oligonucleotide synthesis have been developed, see, for example, M. J. Gait "Oligonucleotide Synthesis, a Practical Approach", IRL Press, Oxford, 1984; J. W. Engels and E.
15 Uhlman, "Gene Synthesis", Angew. Chem. Int. Ed. Engl. (1989) 28:716-724. These methods include the phosphate diester, phosphate triester, phosphite triester and phosphonate methods. Phosphite triester chemistry, which utilizes highly reactive phosphoramidites as starting materials is currently
20 the most favored method of synthesis (R. L. Letsinger, J. L. Finnan, G. A. Heavner and W. B. Lunsford, "Phosphite Coupling Procedure for Generating Internucleotide Links", J. Chem. Soc. (1975) 97:3278-3279; L. J. McBride and M. H. Caruthers, "An Investigation of Several Deoxynucleoside Phosphoramidites
25 Useful for Synthesizing Deoxyoligonucleotides", Tetrahedron Lett. (1983) 24:245-248) Oligonucleotides are most commonly prepared with automated synthesis (Beaucage, et al., Tetrahedron Lett. (1981) 22:1859-1862; U.S. Patent No. 4,458,066). All of the known methods are applicable and will
30 provide molecular building blocks for the supramolecular assembly principle described in this application.

Enzymatic methods for the production of oligonucleotides may also be used to synthesize the polynucleotide moieties of the supramolecular components of the invention. The
35 polynucleotide moieties may also be produced in vivo and subsequently cleaved into complementary single strands by heating, and separated by preparative electrophoresis or

chromatography.

Short oligonucleotides may be coupled together chemically or enzymatically to obtain longer oligonucleotides, see, for example (S. A. Narang, et al., Meth. Enzymol. (1979) 68:90; U.S. Patent No. 4,356,270); N. G. Dolinnaya, N. I. Sokolova, D. T. Ashirbekova and Z. A. Shabarova, "The use of BrCN for assembling modified DNA duplexes and DNA-RNA hybrids; comparison with water soluble carbodiimide", Nucleic Acid Res. (1991) 19:3067-3072).

10

B. Preparation of Effector Molecules

Effector molecules, which contain aliphatic amino, dialkylamino, trialkylamino, thiol, formyl oxirane, α -halogenocarbonyl, isothiocyanato or hydroxysuccinimidyl ester groups of similar, may be coupled with suitably derivatized oligonucleotides using bifunctional spacers. Effector molecules which do not contain groups mentioned above may be activated so that they contain at least one of these groups for coupling. Groups that can be activated for coupling, include: carbon-carbon double and triple bonds, halogen, carbonyl, carboxyl and hydroxyl.

The amino acid residue sequence of proteins may altered through well-known genetic techniques engineering techniques to as to produce non-naturally occurring proteins having the desired biological functions of a corresponding naturally occurring protein, but adapted for coupling to nucleic acid moieties. For example, addition of a cysteine residue, either through substitution or inserting, may add a free third group for coupling to a nucleic acid moiety.

30

C. Attachment of Nucleic Acids to Effector Molecules

Effector molecules may be attached to nucleic acids by numerous methods, including:

1. The molecular moiety is first attached to a solid support and is used as a linker for oligonucleotide synthesis. When oligonucleotide synthesis is completed the molecular moiety is detached from the solid support so that it remains

covalently coupled with the oligonucleotide. An example of this procedure is a Fmoc-protected polypeptide which is first synthesized on a solid support so that it has a terminal free serine hydroxyl group. The oligonucleotide synthesis is
5 started from this hydroxyl group.

2. Molecular moieties other than nucleotides may be incorporated inside the oligonucleotide chain during the synthesis so as to provide functional groups for coupling to nucleic acids. For example, if these molecular moieties have
10 at least two hydroxyl groups, one of which is free and another which is protected by dimethoxytrityl group, then conventional oligonucleotide synthesis methods can be used to produce a nucleic acid that may readily be coupled to an effector molecule.

3. As a last step of the oligonucleotide synthesis a molecular moiety having a suitable functional group for coupling may be attached at the end of the oligonucleotide chain. Again, if this molecular moiety has at least one hydroxyl group, it can be attached as nucleic acid monomer.
15 This approach is already well known in the literature

4. A molecular moiety having a suitable functional group for coupling may be attached after the oligonucleotide synthesis is completed and part or all protecting groups have been removed. Especially molecular moieties attached using
25 methods 1-3 can contain several functional groups which are protected by orthogonal protecting groups. This allows stepwise removal of protective groups and allows regioselective attachment of new molecular moieties.

Methods of attaching enzymes to oligonucleotides that are
30 known to the person ordinary skill in the art may be used to produce the supramolecular components and supramolecular structures of the invention. Descriptions of such techniques can be found in, for example, Jablonski et al. Nucl. Ac. Res. 14:6115-6128 (1986), Ruth DNA 3:123 (1984), Balaguer et al.
35 Anal. Biochem. 180: 50-54 (1989), Balaguer et al. Anal. Biochem. 195: 105-110 (1991), Li et al. Nuc. Ac. Res. 15:5275-5287 (1987), Ghosh et al. Anal. Biochem. 178:43-51 (1989),

Murakami et al. Nuc. Ac. Res. 14:5587-5595 (1989), and Alves et al. Anal. Biochem. 189:40-50 (1988).

In order to covalently couple an oligonucleotide with a effector molecule, the oligonucleotide must contain a functional group which has a high enough reactivity to allow specific reaction at predetermined site. This functionality can be introduced into an oligonucleotide chain during normal automated synthesis, if suitable joint molecules are used. Possible functionalities include amino, dimethylamino, thiol, oxirane and other groups, which are more reactive than functional groups in nucleotides. A different approach is to use biotin-avidin chemistry or another high affinity specific non-covalent interaction. Several means of introducing these groups has already been published in the literature. See, for example, Leary, et al., Proc. Natl. Acad. Sci. USA (1983) 80:4045; Richardson and Gumpert, Nucl. Acid Res. (1983) 11:6167; Lenz and Kurz, Nucl. Acid Res. (1984) 12:3435; Meinkoth and Wahl, Anal. Biochem. (1984) 138:267; Smith, et al., Nucl. Acid Res. (1985) 13:2399, J. M. Coull, H. L. Weith and R. Bischoff, Tetrahedron Lett. (1987) 27:3991-3994; J. Haralambidis, M. Chai and G. W. Tregar, Nucleic Acid. Res. (1987) 15:4857-4876; B. C. F. Chu and L. E. Orgel, Nuc. Acid Res. (1988) 16:3671-3691. In addition to the added functionality of the oligonucleotide strand, a bifunctional spacer molecule is typically used to couple oligonucleotide and a effector molecule. Many of these spacers are well known in the literature and are commercially available.

1. Attachment of Nucleic Acids to Peptides

Peptides and peptide analogues are very commonly used as effector molecules. In order to attach oligonucleotides by normal nucleotide chemistry to a peptide, the peptide should have free hydroxyl groups. Primary hydroxyl groups are preferred. These can be implemented into a peptide by using protected ethanolamine on the carboxyl end and glycolic acid on the amino terminal, instead of an amino acid. As shown in Figure 10, serine moieties can be used to give further

attachment sites along the peptide backbone.

As shown in Figure 11, the peptide effector molecule can be branched and used as a multivalent effector structure. Several other multivalent effector structures are possible such as ethylene glycol dimer, trimer, etc. Ethylene glycol derivatives can be connected to polyalcohol to get multivalent effector structures. In order to fully exploit the present invention, conjugation of several nucleic acid chains to a single effector molecule must be possible. The process of combining nucleic acids with polymeric support and with the use of spacer molecules is well known. Similar chemistry can be used in connection with this invention to combine nucleic acid chains with effector molecules such as proteins or polypeptides.

One method for conjugating several nucleic acid chains to a single effector molecule is described below. The hydroxyl group of 2-(2'-aminoethoxy)ethanol (AAE) is first protected by t-butyldimethylsilylchloride (TBS). The product is coupled with FMOC-t-BOC-L-lysine. FMOC-group is removed and two FMOC-glycines are attached similarly. FMOC-L-glutaminic acid -t-butyl ester is the next component and will later serve as a branching point (see Figure 13). Peptide chain is extended with two glycines and one lysine. The amino group of the last lysine is reacted with propylene oxide whereby a secondary hydroxyl group is formed. This hydroxyl group is protected with acid and base stable trichloroethoxycarbonyl group (Troc).

A shorter peptide based chain is synthesized by starting with Troc protected 2-(2'-aminoethoxy)ethanol and coupling this with one lysine and two glycines using standard peptide chemistry.

Two peptide chains which are prepared as described above are coupled together by forming an amide bond between the free carboxylic group of glutaminic acid and the end amino group of the glycine in the shorter peptide. The product which has three branches each having one protected hydroxyl group needs manipulation of the protecting groups before it is compatible

with oligonucleotide synthesis.

Once the properly protected spacer is prepared the first preprepared oligonucleotide is coupled with phototriester synthesis with the free primary hydroxyl group (Figure 14).
5 The shortest oligonucleotide is coupled in this stage, whereas the longest oligonucleotide is prepared with automatic synthesizer. The product is not deprotected or detached from the solid support. The synthesis is continued by adding the "trivalent" spacer, which is already coupled with one
10 oligonucleotide. The free secondary hydroxyl group becomes coupled with the oligonucleotide which is still bound with the solid support. Thus the peptide spacer is coupled with two oligonucleotide chains. Dimethoxytrityl protecting group of the third hydroxyl group is removed by acid. The automated
15 oligonucleotide synthesis is continued and the third oligonucleotide chain is constructed. The protecting groups are then removed and the molecule is detached from the solid support.

20 D. Assembly of Supramolecule from Components

The hybridization is performed preferably in a aqueous medium containing various additives. Additives include, but are not limited to buffer, detergent (0.1 % to 1 %) , salts (e.g., sodium chloride, sodium citrate from 0.01 to 0.2 M),
25 polyvinylpyrrolidine, carrier nucleic acid, carrier proteins, etc. Organic solvents may be used in conjunction to water, such as alcohols, dimethyl sulfoxide, dimethyl formamide, formamide, acetonitrile, etc. In addition to concentration of the derivatized oligonucleotides, the temperature can be used
30 the hybridization. The optimum temperature for hybridization is 20 °C below the melting point of the oligonucleotide. This means that the preferred temperature for hybridizing 30-mers is typically 40 - 60 °C. For shorter oligonucleotides the temperature is lower and for longer oligonucleotides it is
35 higher. Oligonucleotides containing large portion of cytidine and guanine have higher melting point than the oligonucleotides containing a lot of adenine and thymidine.

Detailed formulae for calculating the melting temperature of double stranded nucleic acids are well known to the person of ordinary skill in the art. Additionally, melting temperature may readily be calculated using empirical methods.

5

5. Example of Antibody-multienzyme Supramolecule

Two current main strategies for drug development for HIV are finding of reverse transcriptase and HIV protease inhibitors. All four approved AIDS drugs are reverse
10 transcriptase inhibitors. HIV protease inhibitors are also promising as drugs, but the rapid mutation of the viral protease has so far been overwhelming obstacle for the development of a commercial drug.

-Embodiments of the supramolecules of the invention that
15 comprise an HIV-antibody and several digestive enzymes can destroy the virus particle itself. Antibodies have earlier been conjugated with enzymes for drug use (C. Bode, M. S. Runge and E. Haber in "The Year in Immunology 1989-1990". Molecules and Cells of Immunity (J. M. Cruse and R. E. Lewis,
20 Eds.) Vol. 6, Karger Publishing, Basel, 1990). Typically these antibody-enzyme complexes are used to produce active drugs from prodrugs. This embodiment of the invention is particularly advantageous if the drug of interest is highly toxic at therapeutic levels. For example, the drug against
25 cancer can be produced on the surface of the cancer cell and cancer cells are subjected to higher concentration of this drug than other cells.

One strategy is to couple lipid and RNA degrading enzymes to an HIV specific antibody. Although a virus does not have
30 its own metabolism serve as a drug target, a virus is unable to heal itself, if part of the virus is destroyed by externally added catabolic enzymes.

In order these enzymes to have operational freedom, the spacer between the antibody and the enzyme must be of
35 sufficient, e.g., e.g., on the order of 10 nm. In this case virtually the whole surface of the virus is covered. In order to avoid allergic reactions this spacer must be fully

biocompatible, preferably a normal biological component. In addition it should have some rigidity to allow structures in which enzymes and antibodies do not interfere with each other. Because these antibody-enzyme complexes can be complicated
5 structures, a self-assembly would be ideal. Oligonucleotides fulfill all these requirements. Further requirement is that joints connecting enzymes and antibody with oligonucleotides are as small as possible to suppress immunoreaction. These drugs are supramolecular drugs, i.e., noncovalent interactions
10 are important structural factors. Especially complementary hydrogen bonding of oligonucleotides is essential for the assembly and structural integrity.

In Figure 3A is a schematic representation of one possible supramolecule demonstrating this principle. Antibody
15 is in central position and four different enzymes: phospholipase A₂, lipase, cholesterol esterase and ribonuclease A. Phospholipase A₂ can be supplemented or completely replaced by another phospholipase such as phospholipase C. One extra single stranded oligonucleotide is
20 attached with the antibody. This oligonucleotide is complementary with viral RNA and binds viral RNA when virus is disintegrated.

Many viruses, including HIV-1, are covered by a lipid bilayer which it takes from the host cell when it is formed.
25 The bilayer contains phospholipids, triglycerides and cholesterol esters. Accordingly three enzymes specific for these classes of compounds are used to digest the viral lipid bilayer. When the bilayer is hydrolyzed, fatty acids and lysolipids are formed. These digestion products are soluble
30 in blood plasma and may be bound by albumin, which is a scavenger protein to remove free fatty acids and lysolipids. When the protein core of the virus is exposed to plasma it is to be expected that the protein dissolves spontaneously and RNA is released. This process happens when the virus is
35 internalized into a cell. The lipid bilayer fuses with the plasma membrane of the cell and virus becomes unstable and dissolves into the cytoplasm of the cell. No specific

endocytosis mechanism has been observed for HIV. In essence our idea is to induce the dissolution of the virus outside the cell and destroy viral RNA when it is released. In order to promote the breakdown of RNA a short complementary

5 oligonucleotide is attached with the antibody and also ribonuclease A is part of the enzyme palette. Proteinases are not included among the enzymes in our first design, because it is feasible to suppose that the protein effector of the HIV is unstable when exposed. If opposite turns out to be true, it
10 is possible to include some proteinases. However, blood contains inhibitors against many proteinases, especially if proteinases are nonspecific. Some specific endopeptidases as well as carboxypeptidases and aminopeptidases can be used, because they are not inhibited.

15 A similar strategy can be used for cancer therapy and to remove 'plaque' from blood vessels, e.g., to treat atherosclerosis. In each case antibody must be replaced with another antibody or other recognition molecule, which is specific for the target. Also enzymatic composition must be
20 adjusted for each application.

The antibody-multienzyme supramolecule is assembled from oligonucleotide-enzyme conjugates and branched oligonucleotides according to Figure 3(B). In Figure 3(C) depicts two simplified supramolecules, which together can
25 carry same enzymes as the supramolecule in Figure 3(A).

An important consideration in the synthesis is the incorporation of amino or thiol functionalities into a desired point of the oligonucleotide during automated synthesis. Phosphoramidite synthesis is described in 5.1-5.7.
30 Their use in oligonucleotide synthesis is straightforward. By using amino and thiol specific cross-linking agents, the synthesis of branched oligonucleotides is also easily accomplished. The oligonucleotide strands are by A and B and their complementary oligonucleotides are denoted by
35 corresponding underlined letters. Enzymes are attached into either 3' or 5'-terminus of the oligonucleotide, which contains an amino group. This kind of coupling of

oligonucleotides and proteins is a standard practice in biochemical conjugation. Antibody is attached into the center of a oligonucleotide chain containing an aliphatic amino group in that position.

5 After molecular building blocks are synthesized, the final step is a self-assembly of a supramolecule. This relays on the pairwise complementarity of the oligonucleotide strands in the components, which are designed to bind together. In principle, the components contain the complete information of
10 the structure of the final supramolecule and a simple mixing of the component molecules will produce the wanted product. However, in order to make certain that the assembly proceeds as designed, the stepwise process is to be preferred.

15 Preparation of the antibody-multienzyme supramolecule

Abbreviations: Aminopropanol, AP; 2-Cyanoethyl N,N-diisopropylchloro phosphoramidite, CEDIPCPA; Dichloromethane, DCM; Di-isopropyl ethyl amine, DIPEA; Fluorenylmethoxycarbonyl, Fmoc;

20 Fluorenylmethoxycarbonylchloride, FMOCcl; Methanol, MeOH; Monomethoxytrityl, MMT; Monomethoxytritylchloride, MMTcl; Serinol, SER; Tetrahydrofuran, THF; Triethylamine, TEA.

5.1. N-Monomethoxytrityl Aminopropanol (MMT-AP)

25 MMTcl (1.54 g) in 10 ml of DCM was added to a solution of AP (1.5 ml) in 5 ml of DCM. The reaction mixture was 24 h at + 4 °C. 10 ml of DCM was added and the mixture was washed twice with 10 ml of 5 % NaHCO₃ and with 5 ml of water. The DCM phase was dried with solid NaHCO₃. The solution was
30 concentrated into 5 ml in vacuo and applied to 40 g silica column, which was eluted with 300 ml of DCM/TEA 200:1, 300 ml of DCM/EtOAc/TEA 200:2:1 and 200 ml of DCM/EtOAc/TEA 100:2:1. 1.32 g of pure MMT-AP was obtained.

5.2. N-Monomethoxytrityl aminopropyl cyanoethyl N,N-diisopropylphosphoramidite (MMT-AP-CEDIPPA)

To a solution of MMT-AP (0.42 g) in 8 ml of DCM was added 475 μ l of EDIPA and 0.30 g of CEDIPCPA in 2.5 ml of DCM. After 10 min the reaction mixture was applied directly to 10 g silica column. The column was eluted with DCM/EtOAc/EDIPA 98:1:1. Fractions of 6 ml were collected. The product was in fractions 3 and 4. The yield was 0.44 g.

This product was used in oligonucleotide synthesis.

5.3. N-Fluorenylmethoxycarbonyl aminopropanol (FMOC-AP)

FMOCCL (1.55 g) in 10 ml of THF was added into a solution of 0.90 g of AP in 40 ml of water. After 30 min stirring the reaction mixture was extracted with 20 ml of DCM. The DCM solution was washed twice with 10 ml of water and dried with $MgSO_4$. The solvent was removed with a rotary evaporator and the residue was dissolved into 14 ml of EtOH and 14 ml of water was added. The small precipitate was filtered off and the solution was put into a refrigerator. After 20 h the precipitate was separated by filtration. The yield was 1.22 g.

5.4. N-Fluorenylmethoxycarbonyl aminopropyl cyanoethyl N,N-diisopropylphosphor-amidite (FMOC-AP-CEDIPPA)

FMOC derivative was done exactly as MMT analog in Example 2 using 0.30 g FMOC-AP. Also purification was done similarly. The product was in fractions 3-8. Fractions 3-7 contained 0.41 g product.

This product was used in oligonucleotide synthesis.

5.5. N-Fluorenylmethoxycarbonyl serinol (FMOC-SER)

FMOCCL (1.55 g) in 10 ml of THF was added into a solution of 0.54 g of SER in 30 ml of water and 8 ml of 1.5-M Na_2CO_3 . After 30 min stirring the reaction mixture was extracted with 20 ml of EtOAc. The EtOAc solution was washed twice with 10 ml of water and dried with $MgSO_4$. The solvent was removed with a rotary evaporator and the residue was dissolved into a

mixture of 5 ml of EtOH and 30 ml of DCM. The product crystallized in +4 °C. Yield was 1.12 g.

5.6. N-Fluorenylmethoxycarbonyl O-dimethoxytriphenyl serinol (FMOC-DMT-SER)

5 FMOC-SER (1.12 g) was dissolved into 6 ml of pyridine and 0.68 g of solid DMTrCl was added. The reaction mixture was put into +4 °C. After 20 h 20 ml of water was added and the oily layer was washed with 5 ml of water and dissolved into 10 ml of EtOAc and the solvent was removed in vacuo. The residue (1.76 g) was fractionated in 28 g silica column, which was eluted with DCM/EtOAc/MeOH/TIPEA 98:1;0.2:0.5 and 96:4:1:0.5. Yield of pure product was 0.72 g.

15 5.7. N-Fluorenylmethoxycarbonyl O-dimethoxytriphenyl serinyl cyanoethyl N,N-diisopropylphosphoramidite (FMOC-DMT-SER-CEDIPPA)

FMOC-DMT-SER derivative was produced essentially as described for the phosphoramidite in Example 5.2 using 0.65 g FMOC-AP. The product was purified similarly. The desired reaction product was found in fractions 4-9. Fractions 5-8 contained 0.82 g product. TFMOC-DMT-SER may also be synthesized by first protecting serinol with DMT and then with FMOC. This variation allows also acylation of the amino group of serinol with carboxylic acids carrying various other functionalities, such as protected amino or thiol groups and biotin.

The desired product was used in automated synthesis to introduce aliphatic amino group in the position of 20 in a 51-mer.

30

5.8. Automated Synthesis of Oligonucleotides

The following oligonucleotides were synthesized by automated synthesis:

35 A 3' TGGAGATGGGGCACCATGCTX5'
(SEQ ID NO:1)

B 3' AGCATGGTGCCCCATCTCCAYAGTCACAGCACAGCACTAATAACAAGAAAS'

(SEQ ID NO:2)

C 3' TYTTTCTTGTTATTAGTGCTGTGCTGTGACT5'
(SEQ ID NO:3)

D 3' TGGTCCTCTAGAGTTTTTGAGGGTX5'
5 (SEQ ID NO:4)

E 3' CCCCTCAAAAACCTCTAGAGGACCAYTTATCTGGGCAGGCTGAGCTCGGT5'
(SEQ ID NO:5)

F 3' TYACCGAGCTCAGCCTGCCCAGATAA5'
(SEQ ID NO:6)

10 X represents MMT-AP-CEDIPPA (5.2) and Y represents FMOC-DMT-SER-CEDIPPA (5.7). Analogous amidites may also be to introduce aliphatic amino groups.

5.9. Purification of Monoclonal Antibody

15 Anti gp41/160 (antibody IAM3D6) supernatant had a concentration of 315 mg/l. It was purified in 160 ml portions in Protein A Sepharose Fast Flow 5 ml column. The supernatant was buffered with 40 ml of 0.2-M Na_2HPO_4 . After feeding the supernatant into the column, the column was washed with 120 ml
20 of 0.1-M Na_2HPO_4 . The antibody was eluted off the column with 0.1-M citric acid and neutralized immediately with 3-M KOH. The antibody solutions were stored at -18 °C.

5.10. Acetylated Protein A Sepharose Gel

25 Protein A Sepharose was packed into 1.5 ml column. It was saturated by eluting with a solution containing 50 mg of monoclonal antibody (Anti gp41/160 IAM3D6). The column was washed with 0.1-M Na_2HPO_4 buffer (15 ml) and eluted 10 ml 1 mM acetyl N-hydroxy succinimide solution in DMF/water 1:9. The
30 antibody was removed by 0.1-M citric acid. The acetylated Protein A Sepharose was used to couple antibody with nucleotides and in the final assembly of the supramolecule.

5.11. Coupling of Oligonucleotide with Antibody

35 A solution of antibody (40 mg/25 ml water) was eluted through the column containing 1.5 ml acetylated Protein A

Sepharose. The Sepharose was washed with 3 ml of 0.1-M Na_2HPO_4 and taken out of the column to perform a bath reaction with derivatized nucleotide.

5 Oligonucleotide 2 (10 mg, 0.5 μmole), comprising two equal 30-mers bound together by an amino group containing joint, was dissolved into 1 ml of 0.1-M NaHCO_3 and 50 μl of 1-M solution of bis(hydroxysuccinimidyl) glutarate in acetonitrile was added. After one hour the water solution was
10 extracted twice with 1 ml of EtOAc and the solution was dialyzed 2 h against 0.1-M NaHCO_3 . The activated nucleotide was added into a slurry of Sepharose. The mixture was stirred six hours and packed into a column. The antibody coupled to the nucleotide was eluted off the column with 0.1-M citric
15 acid. Antibody-oligonucleotide conjugate was fractionated in a Sephadex G-25 column and antibody connected with oligonucleotide was collected.

5.12. Coupling of Oligonucleotide with Enzymes

20 Oligonucleotide 1 (20 mg, 2 μmole), which was contained aliphatic amino group at 5'-position was dissolved into 2ml of 0.1-M NaHCO_3 and 400 μl of 1-M solution of bis(hydroxysuccinimidyl) glutarate in acetonitrile was added. After one hour the water solution was extracted twice with 1
25 ml of EtOAc. The solution was dialyzed 2 h against 0.1-M NaHCO_3 and 0.5 ml aliquots of this solution were added into the following enzyme solutions:

- a. 10 mg phospholipase A_2 in 1 ml of water.
- 30 b. 40 mg lipase in 4 ml of water.
- c. 10 mg ribonuclease in 1 ml of water
- d. 30 mg carboxypeptidase in 3 ml of water

5.13. Assembly of the supramolecule

35 Antibody connected with oligonucleotide was eluted through a acetylated Protein A Sepharose column (1.5 ml) so that the column was saturated with antibody. The column was

thermostable at + 40 °C and phospholipase-oligonucleotide conjugate solution (twice the equivalent amount) was circulated through the column and UV-flow cuvette. When UV-absorption at 280 nm was decreased into half the ribonuclease
5 A-oligonucleotide conjugate was circulated similarly through the column. Generally about two hours was needed for a complete reaction. The supramolecule was eluted off the column by 0.1-M citric acid and neutralized immediately with 1-M KOH. The other supramolecule depicted in Figure 3(C) was
10 prepared similarly.

6. Design Of Supramolecule For Capturing Virus Particles

This example describes the design of a supramolecular assembly that is capable of surrounding a comparatively large
15 particle, e.g, a virus. First, a structure, which is capable of performing the desired function, is designed and the geometrical features are fixed. Then chemical and physical features are chosen based on the application. Hydrophilicity, hydrophobicity, acidity, alkalinity, charge transfer, etc., is
20 mapped onto the structure. This designed structure may be visualized as a single molecule, although in many instances the synthesis of this molecule would be difficult to achieve at a reasonable yield. In such embodiments, supramolecular retrosynthesis is performed, i.e., the structure is broken
25 down into small molecules, which are capable via self-assembly of forming the original structure. The supramolecular assembly produced in this manner is not identical with the molecule represented as a schematic in the figures; however, the important characteristics, i.e., geometry and chemical and
30 physical properties listed above, remain the same.

Supramolecular retrosynthesis does not try to retain the original molecular structure intact, but tries to retain all the important chemical and physical properties of the desired structure.

35 Another retrosynthetic cycle can be performed for the molecules obtained in the previous retrosynthesis to obtain smaller molecular building blocks. Finally, molecules are

obtained that can be designed and prepared easily. In the design example given below, there are two retrosynthetic cycles.

Many viruses have an icosahedral shape. Such a virus can be covered by an icosahedral and assembly designed according to this invention. This process is demonstrated stepwise in Figures 5 A-E. Dimensions referenced are taken from HIV (human immunodeficiency virus), but the same principles apply to any virus. In this example, polypeptides and oligonucleotides are used, because synthetic methods are available for their high yield synthesis. As synthetic methods further develop, analogues or completely artificial supermolecular systems can be made using the same design and construction principles offered by the invention.

Each edge of HIV is about 80 nm. In preliminary design we suppose that three amino acid or nucleic acid residues are needed per nanometer. The circles in Figure 4 represent cyclic polypeptides containing enough lysine so that five polylysine chains can be attached. These polylysines are denoted by zig-zag lines in Figure 4. Polylysine should contain about 200 residues in order to cover whole edge. Onto the other end of each polylysine chain is coupled another cyclic peptide that has four nucleotides attached. These nucleotide strands are denoted by a wavy line in Figure 4. Two of these oligonucleotides are the same, for example, oligonucleotide 1 ($n=p=1$, $i=100$). Two others are mutually complementary, but they are bound to the cyclic peptide so that coupling occurs easily between neighbors, but not intramolecularly. Thus, they form a pentagon shaped double helix. Figure 4 shows single stranded oligonucleotide is bound by polylysine. Another molecule is designed using the same principles, but instead of oligonucleotide 1, the single stranded oligonucleotide is now 1. When either of these molecules encounters a virus, which has a negatively charged surface, polylysine is Coulombically associated with the virus. Simultaneously, a negatively charged oligonucleotide (e.g., 1 or 1) is released from the polylysine. When a

complementary capping molecule is associated with the virus, the complementary oligonucleotides (1 and 1) combine to close the cage from which the virus can not escape.

The molecules shown in Figures 4-5 and described above would be incredibly difficult to synthesize. However, by designing a supramolecular constructed from smaller components, synthesis of a virus capturing molecule is made possible.

Figure 6 demonstrates how the analogous structure for the large molecule in Figure 4 can be prepared using smaller molecules. These smaller molecules are shown separately in Figure 6. Thus, in this approach six different compounds are needed to get the overall structure, which is same as that of the molecule in Figure 4. Four of these are relatively simple, because in each two oligonucleotides are connected to a spacer, which can be polypeptide. Two molecules in the upper part of Figure 4 are still relatively complicated because in both cases five nucleotides are connected to a cyclic spacer, which can be a cyclic peptide. These oligonucleotides are denoted by (3,3,3,3,3) and (1,1,8,2,8). In these notations only free single stranded oligonucleotides are listed. These structures can be synthesized by attaching each type of oligonucleotide needed to a short peptide, for example, pentapeptide Gly-Ala-Ser-Ala-Gly which is otherwise protected but the hydroxyl group of serine is free. Nucleotide is connected with this hydroxyl group using normal phosphate coupling. Then, using peptide synthesis methods, these pentapeptides connected to a specific oligonucleotides are coupled in a desired order. Closing the cycle makes the molecule more symmetric, but is not essential for the supramolecular assembly or the function of this assembly in most cases.

There is a further possibility of assembly of cyclic structures containing five oligonucleotide chains by using the general principles of this application. This second step of supramolecular retrosynthesis is demonstrated in Figure 8. Both of these cyclic structures can be assembled from five

smaller molecules. For (3,3,3,3,3) these molecules are twice (3,1,2) and once (3,2,4), (3,2,4), (3,1,2) and for (1,1,8,7,8) these are (2,1,6), (6,1,5), (5,8,4), (4,7,3), (3,8,2). In these notations it is immediately clear that molecule (2,1,6) combines with the molecule (6,1,5), because complementary nucleotides are written last and first, respectively. Looking at the whole sequence of five molecules indicates that the notation starts with nucleotide 2 and ends with 2. This means that these ends will bind together and form a pentagon. After assembly, a supramolecule is obtained which has the same overall shape as two molecules in upper part of Figure 7. These supramolecules are still denoted by listing only their single stranded oligonucleotides, because this is important for further assembly and is sufficient for purposes of this application. The symbols are (3,3,3,3,3) and (1,1,8,7,8). These supramolecules also function similarly in further assembly of the structure, which has the same shape as the molecule in Figure 4. This demonstrates that almost any structure can ultimately be created from molecules which has a spacer or a molecular moiety having an active role in the final assembly connected to two or three oligonucleotides. The spacer can be a very small molecule or it can be a large molecule. The spacer can actually be a DNA strand.

Supramolecular assemblies are preferably prepared in an aqueous environment, although some embodiments may be assembled in organic solvents. When effector molecules are lipophilic, the Langmuir-Blodgett technique may be utilized. Stepwise assembly is often advantageous. For example, the cyclic structures (3,3,3,3,3) and (1,1,8,7,8) in Figure 8 are assembled separately. These two structures can be stabilized internally by cross-linking their double helices. This cross-linking can be performed in a highly selective manner. By cross-linking, both of these supramolecular assemblies become covalent molecules. Cross-linking is not essential, but can be advantageous, because it increases thermal stability. After first assembly and possible cross-linking, the product can be purified. Purification as well as cross-linking is to

be recommended, if the same oligonucleotide is used in several different places.

During the second assembly step (3,4) and (4,7) (see Figure 6) are added to (3,3,3,3,3) to give (7,7,7,7,7). In the third assembly step the product (7,7,7,7,7) and (1,1,8,7,8) are combined to form 10*(1,8). The fourth assembly step is the formation of a pentagon by adding (8,9) and (8,9) to give 10*(1). The fifth and final assembly step is adding single stranded oligonucleotide 1, and the end product is 10*(1). After each step, cross-linking or purification or both can be performed depending on the final requirements regarding quality of the product. The complementary supramolecule 10*(1) is prepared similarly.

- If necessary, the cage surrounding the virus can be made more dense using the principles of this application. The number of molecules needed is then correspondingly larger.

DNA double helix is thermally unstable and cross-linking may be required for stability. One possible approach is shown in Figure 12. The last amino acid residue in the spacer is lysine and a complementary DNA strand contains an alkylating group, which binds preferentially with the amino group of lysine, because it is the most nucleophilic of the functional groups in this assembly. Thus, perfect chemical control can be maintained also in the cross-linking process, although this is not always necessary and more random cross-linking methods can be used. Incorporating photoactivatable groups, like azido adenosine or bromo- or iodo uridine, into oligonucleotide chains allows photochemical cross-linking, which is site specific. Also use the 3-thioribose in oligonucleotide and cysteine in the peptide spacer allows formation of disulphide bridges.

Incorporation by Reference

All patents, patents applications, and publications cited are incorporated herein by reference.

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-
5 described makes for carrying out the invention which are obvious to those skilled in the field of molecular biology, organic chemistry, or related fields are intended to be within the scope of the following claims.

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CLAIMS

What is claimed is:

1. A supramolecule comprising:

- 5 (i) a first supramolecular component, the component comprising a first effector molecule covalently joined to a first nucleic acid,
- 10 (ii) a second supramolecular component, the component comprising a second effector molecule covalently joined to a second nucleic acid, wherein the second nucleic acid comprises a region of at least partial complementarity to the first nucleic acid, wherein the first nucleic acid is in a base pairing relationship with the second nucleic acid.

15 2. A supramolecule according to Claim 1, wherein the first effector molecule is an antibody.

20 3. A supramolecule according to Claim 2, wherein the second effector molecule is an antibody.

4. A supramolecule according to claim 2, wherein the second effector molecule is an enzyme.

25 5. A supramolecule according to claim 1, wherein the effector molecule is a ligand, wherein said ligand is a member of a ligand-receptor pair.

30 6. A supramolecule according to claim 2, wherein the antibody is specific for a viral protein.

7. A supramolecule according to claim 1, the supramolecule further comprising,

- 35 (iii) a third supramolecular component, the component comprising a third effector molecule covalently joined to a third nucleic acid, wherein the third nucleic acid comprises a region of at least partial complementarity to the first nucleic

acid or the second nucleic acid, wherein the third nucleic acid is in a base pairing relationship with the second nucleic acid or the first nucleic acid.

5 8. A supramolecule according to Claim 2, wherein said antibody is specific for a viral protein.

9. A supramolecule according to Claim 2, wherein said antibody is specific for a cancer cell marker.

10

10. A supramolecule according to Claim 2, wherein said antibody is specific for a molecule characteristic of atherosclerotic plaque.

15 11. A supramolecule according to claim 4, wherein said enzyme is selected from the group consisting of glycosidases, phospholipases, lipases, cholesterol esterases, and nucleases.

20 12. A supramolecule according to claim 4, wherein said first effector molecule is an antibody specific for a viral protein.

25 13. A supramolecule according to claim 5, wherein said first ligand comprises CD4.

14. A supramolecule according to claim 4, wherein said first effector molecule is an antibody specific for a cancer cell marker.

30

15. A supramolecule according to claim 4, wherein said first effector molecule is an antibody specific for a molecule characteristic of atherosclerotic plaque.

35

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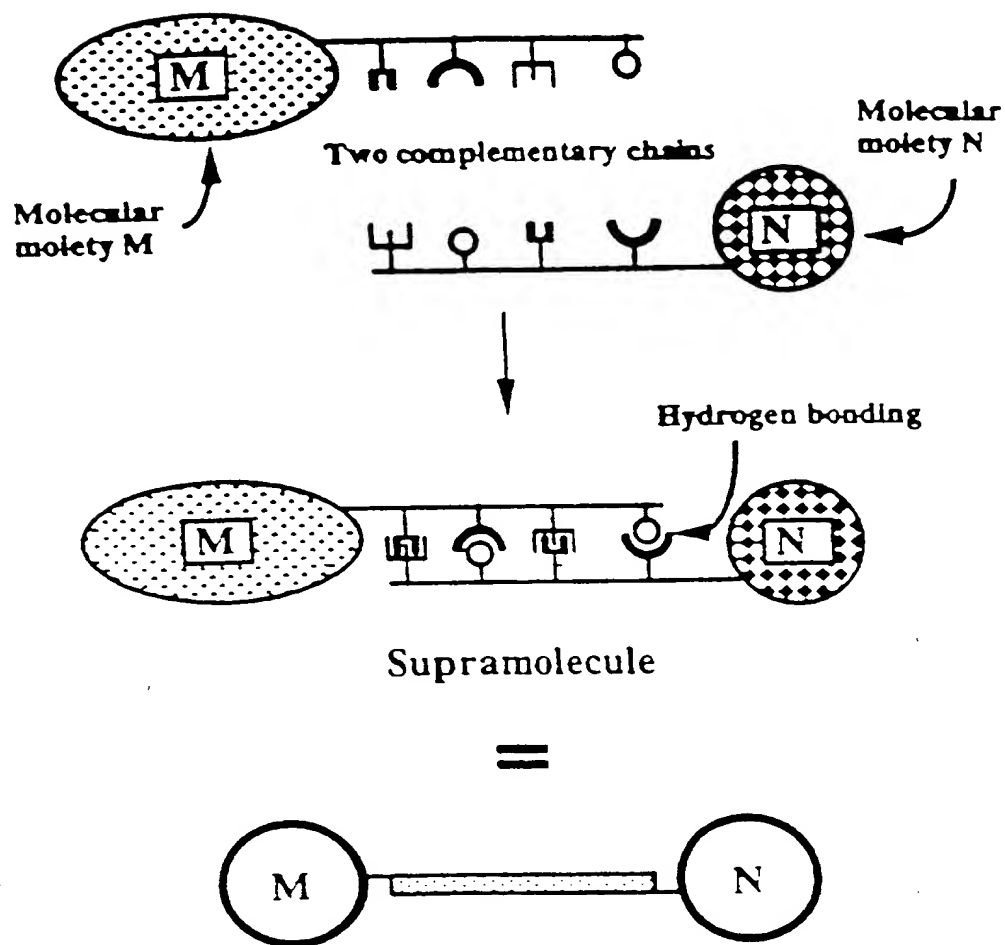


Fig. 1

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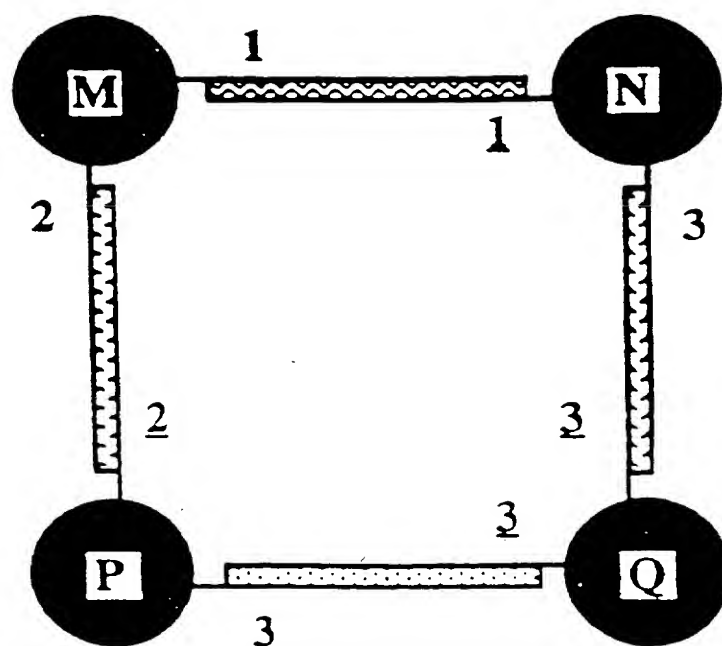
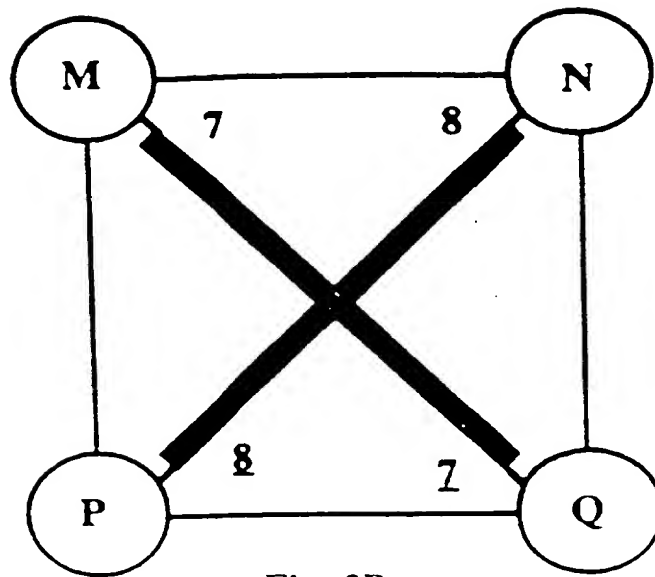


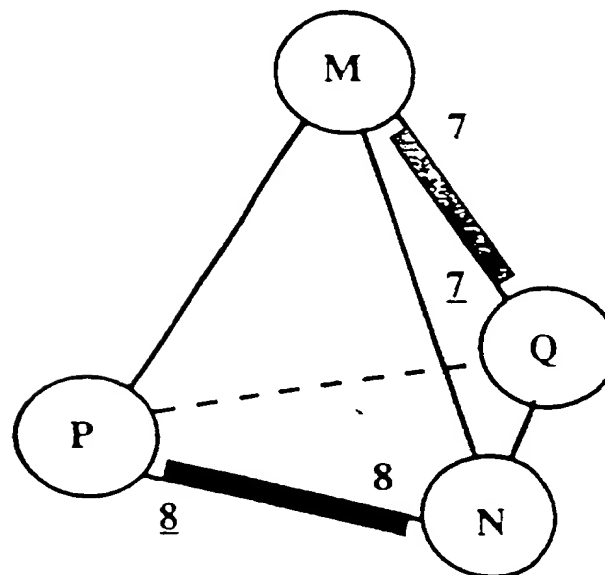
Fig. 2A

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$$j = 1.41 * i$$

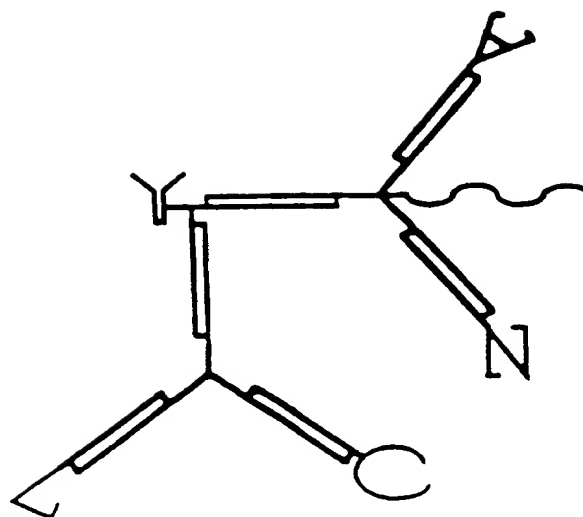
Fig. 2B



$$j = i$$

Fig. 2C

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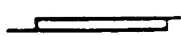
Antibody

Phospholipase A₂

Lipase



Cholesterol esterase



Double helix oligonucleotide



Single stranded oligonucleotide

Fig. 3A

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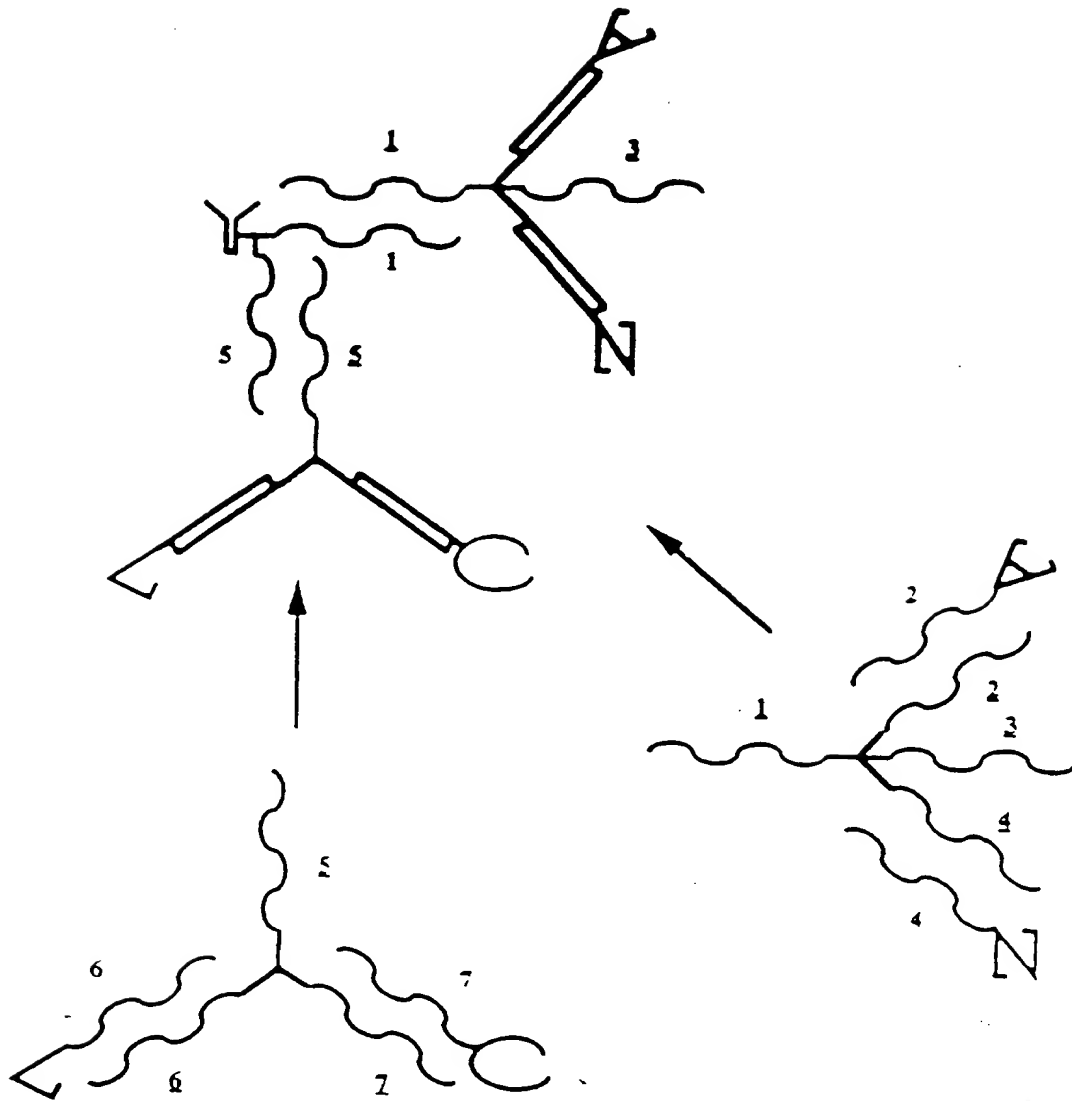


Fig. 3B

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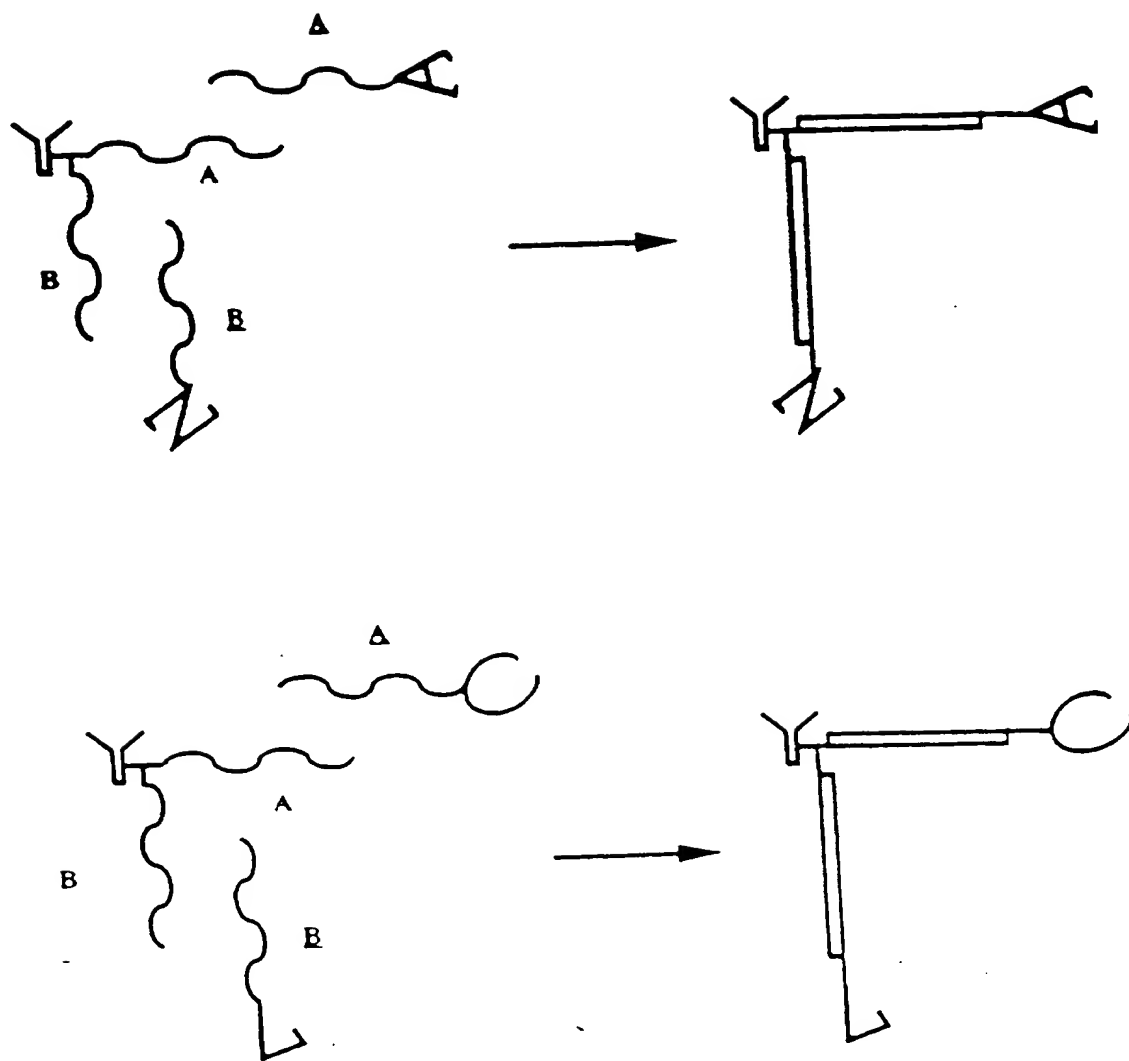


Fig. 3C

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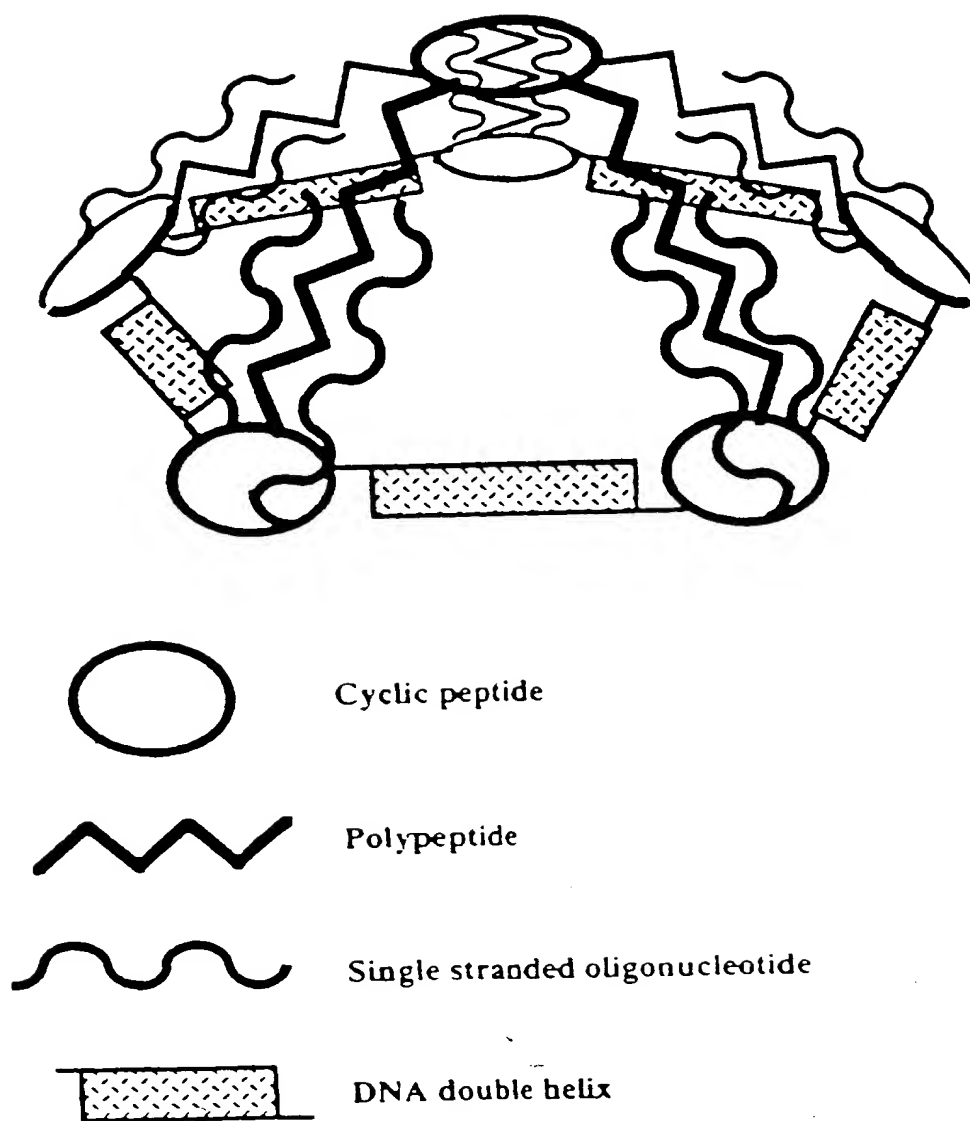


Fig. 4

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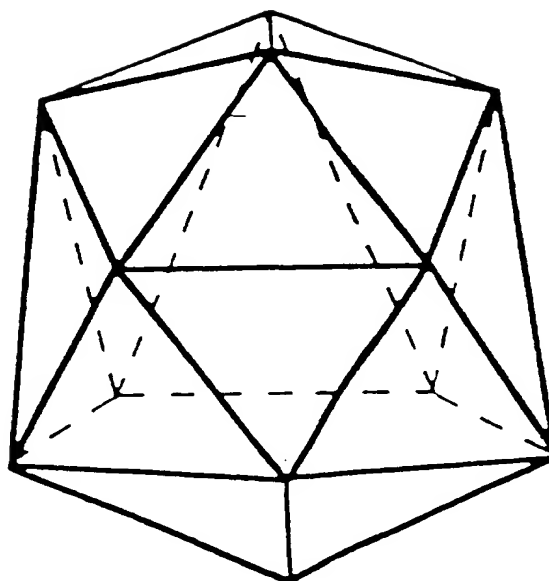


Fig. 5A

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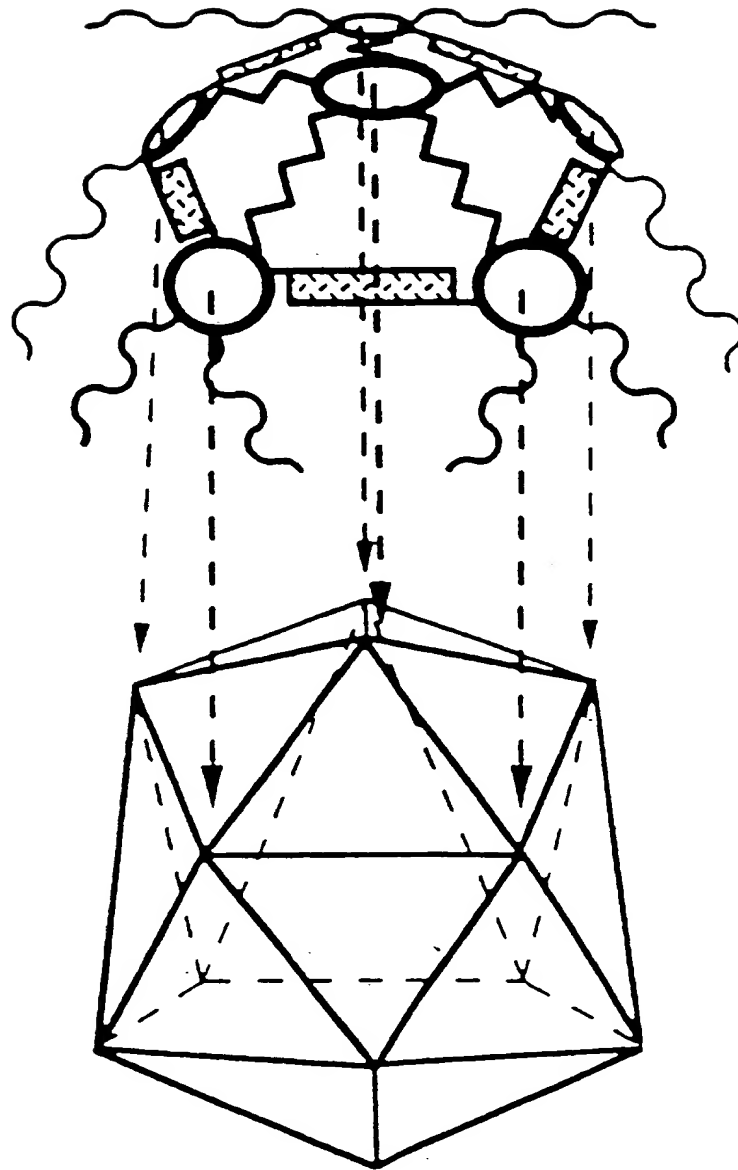


Fig. 5B

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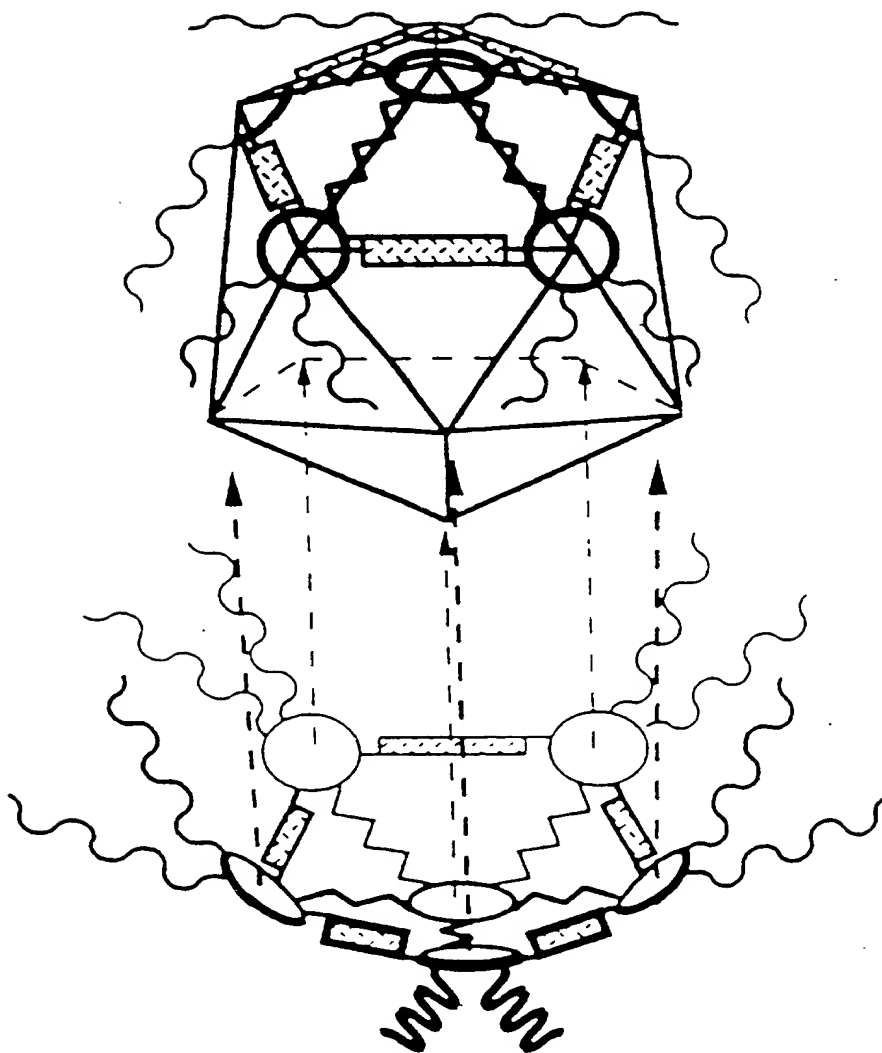


Fig. 5C

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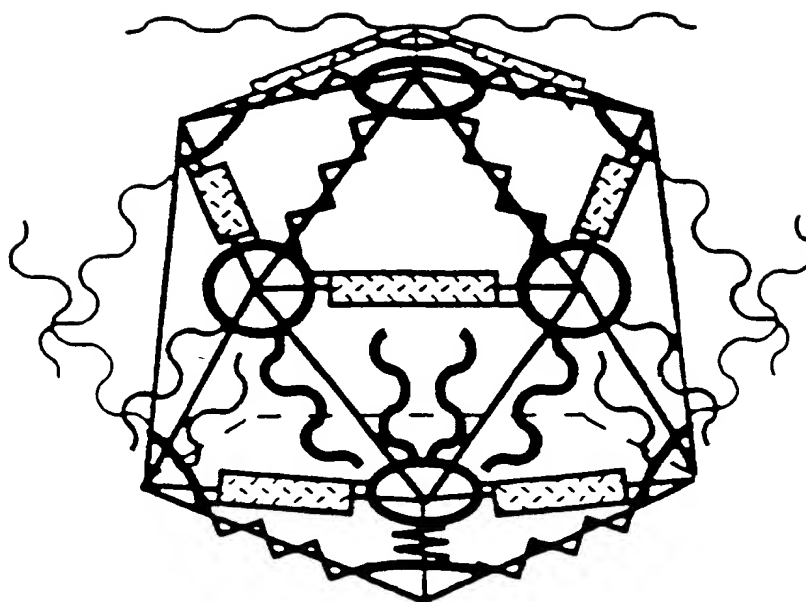


Fig. 5D

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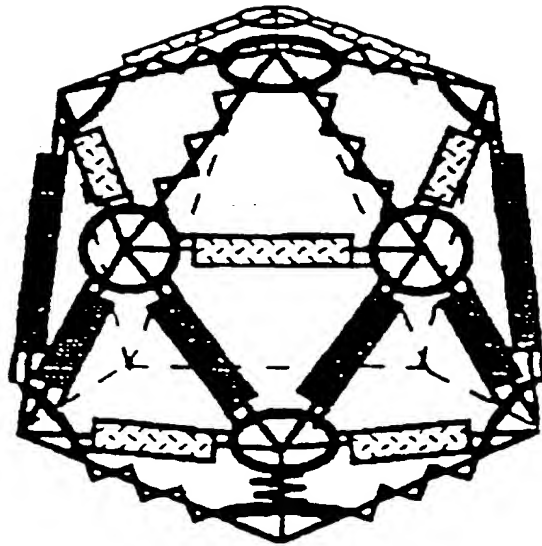


Fig. 5E

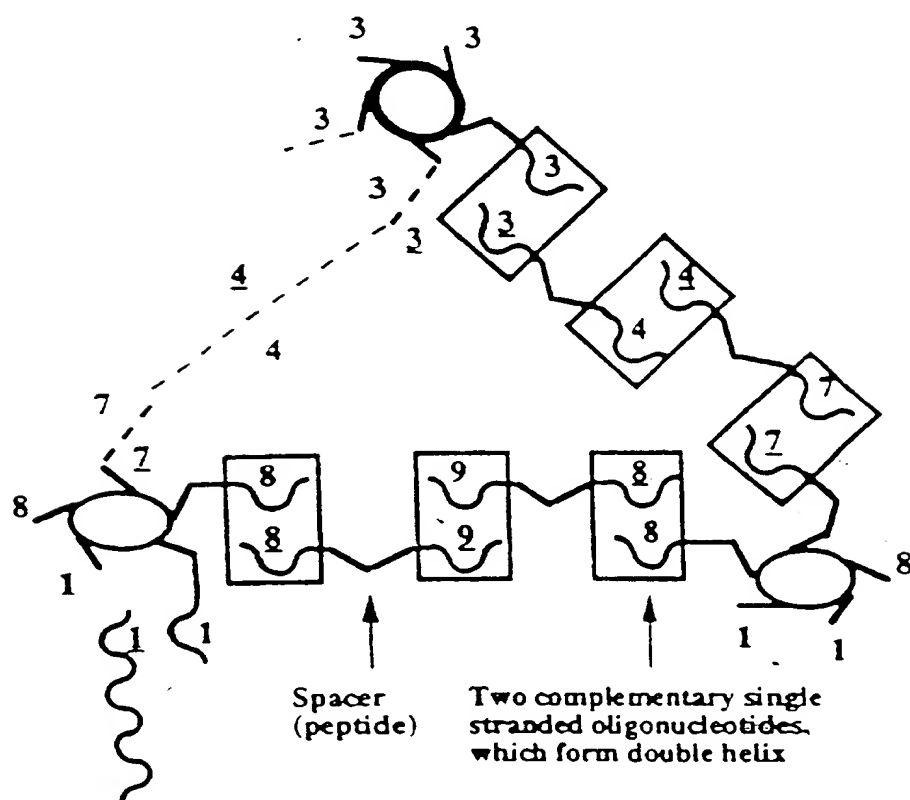
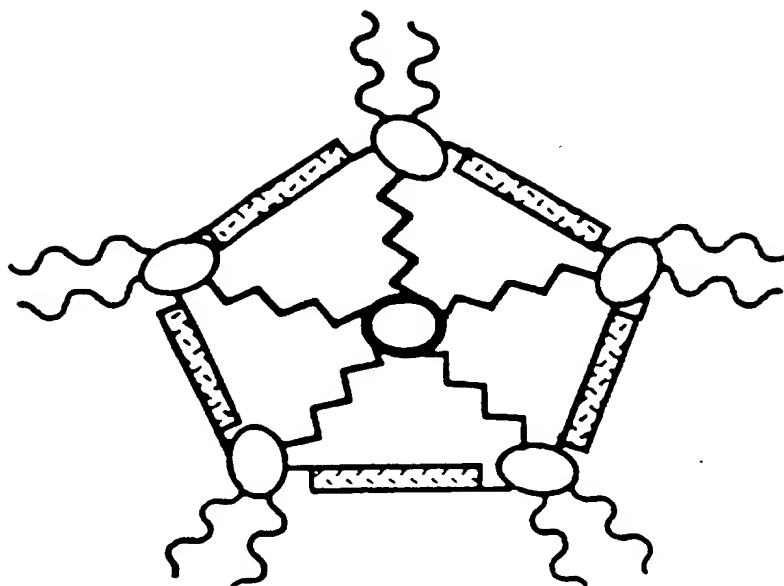
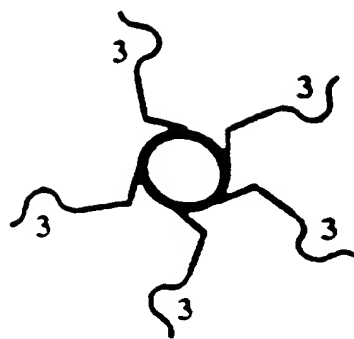
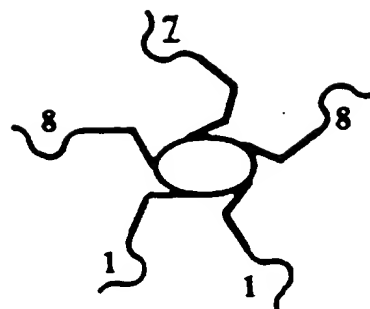


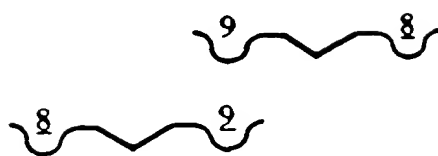
Fig. 6



(3,3,3,3,3)

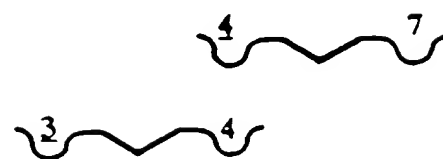


(1,1,7,8,8)



(8,2)

(9,8)



(3,4)

(4,7)

Fig. 7

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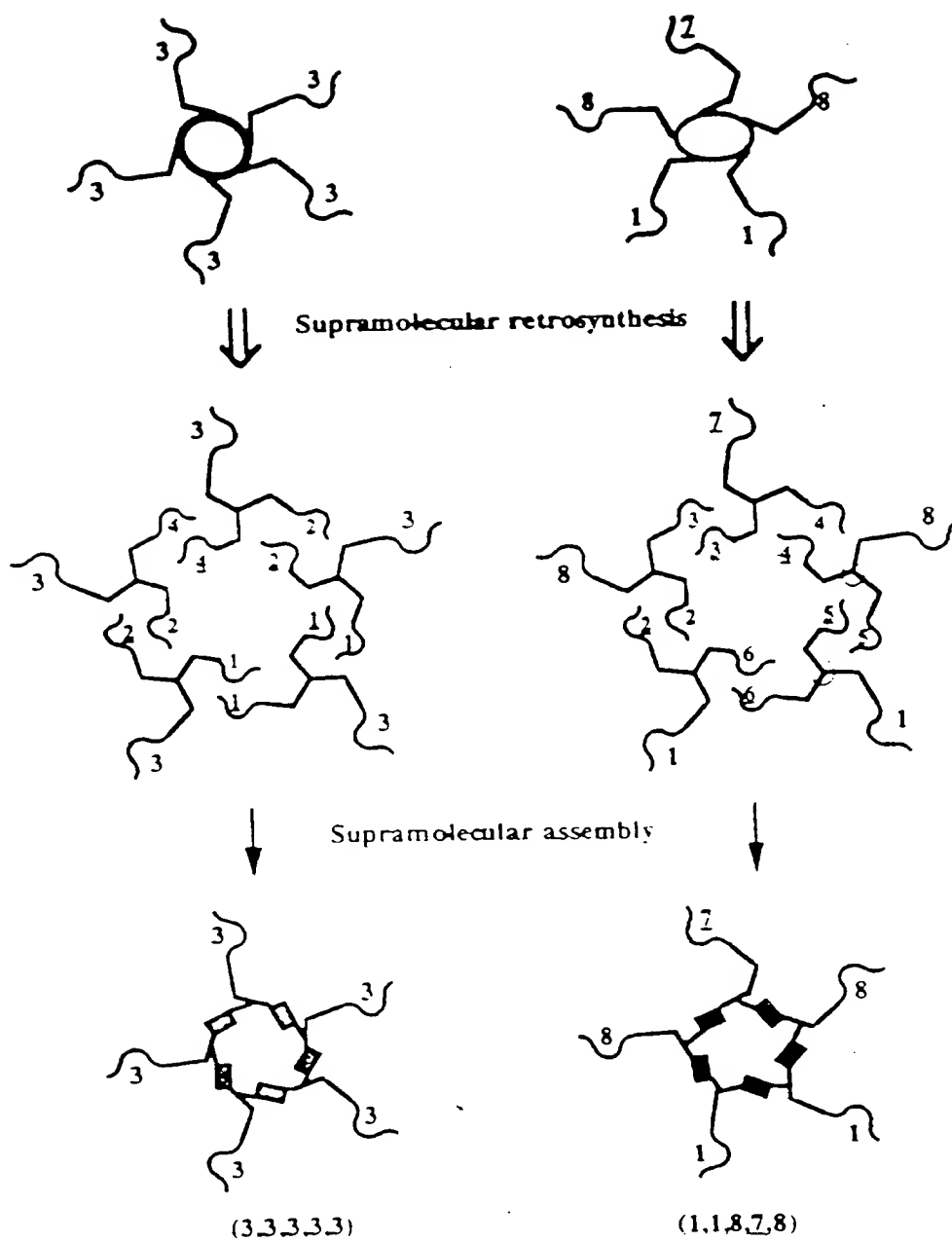


Fig. 8

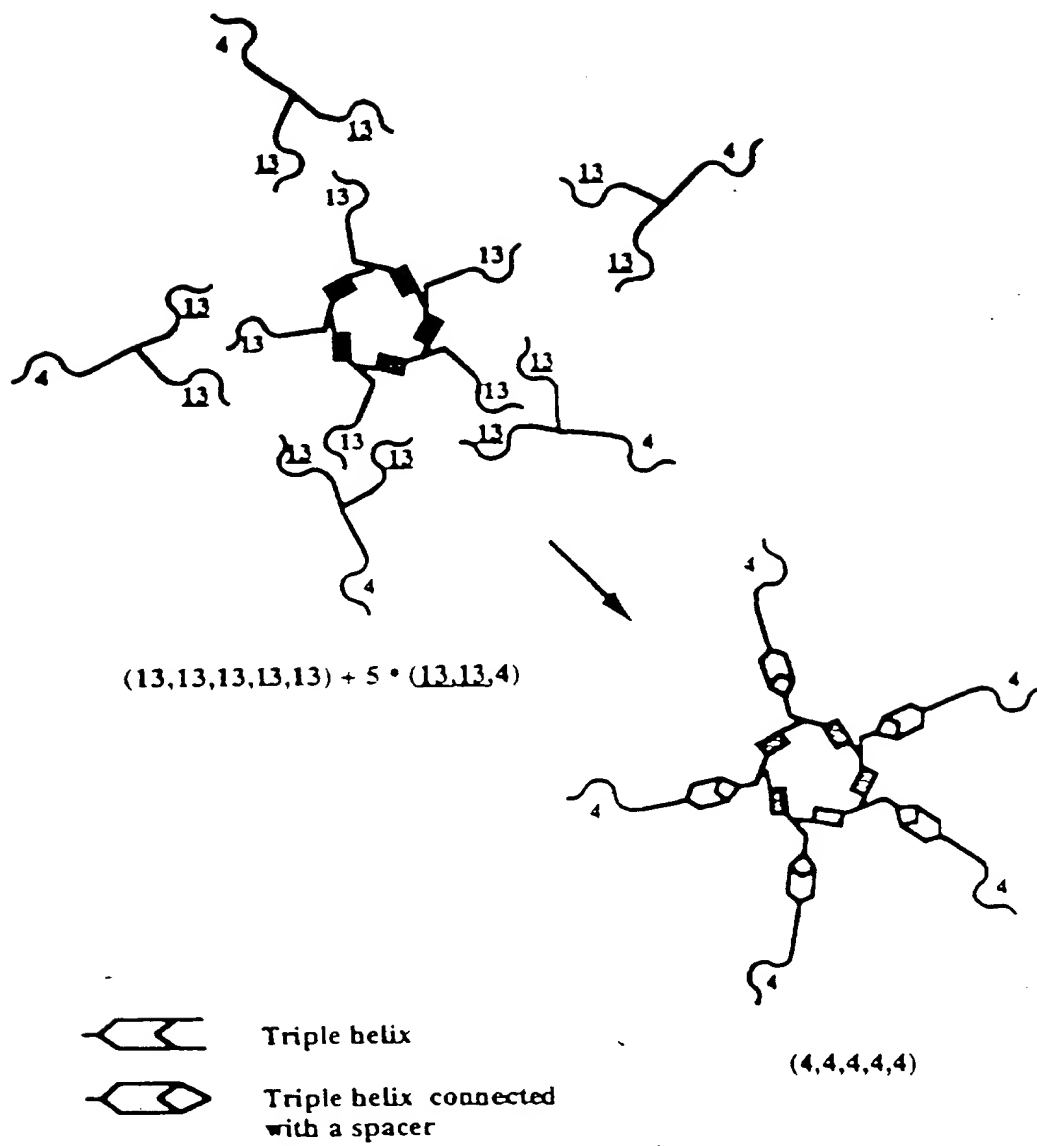


Fig. 9

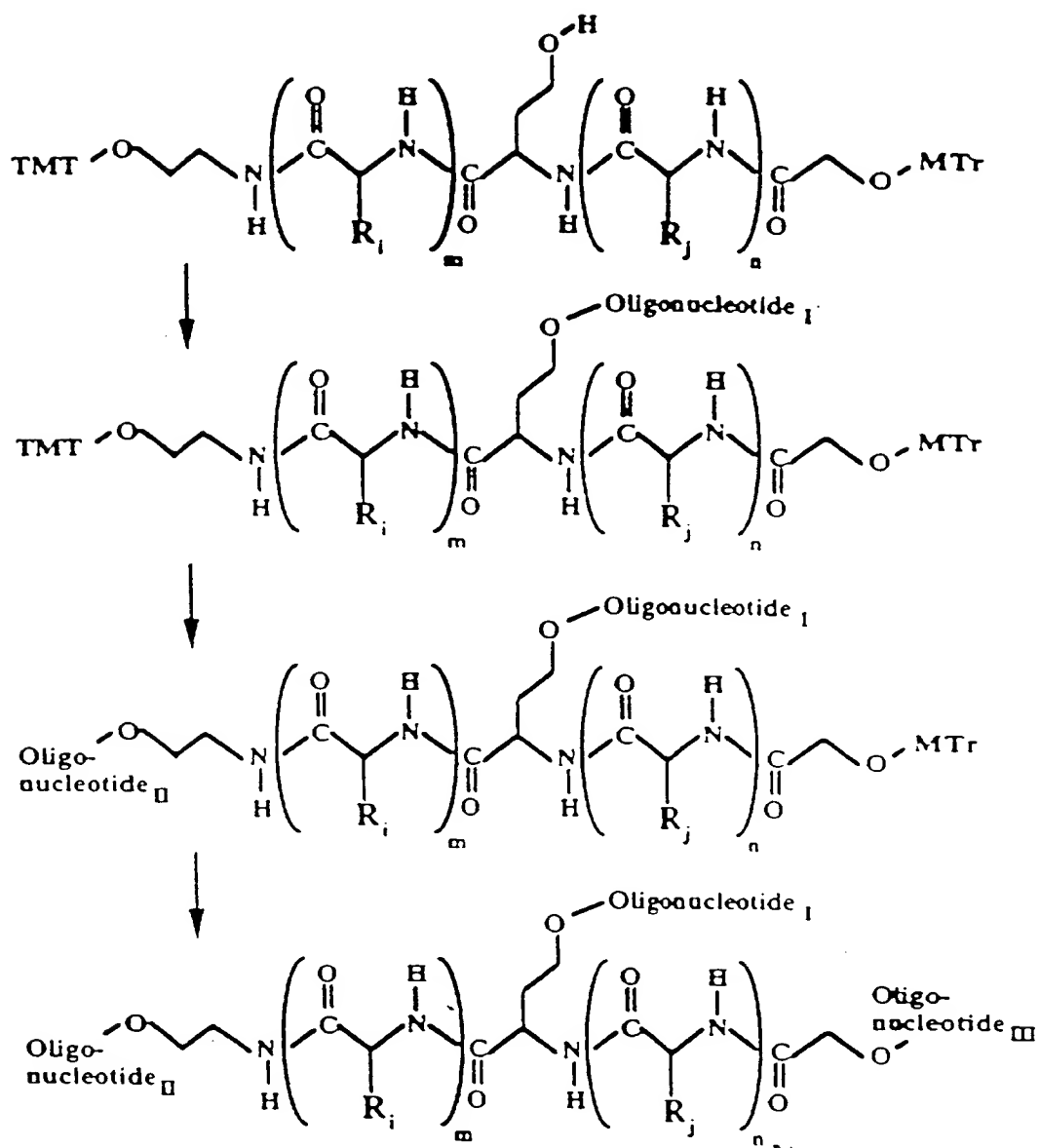


Fig. 10

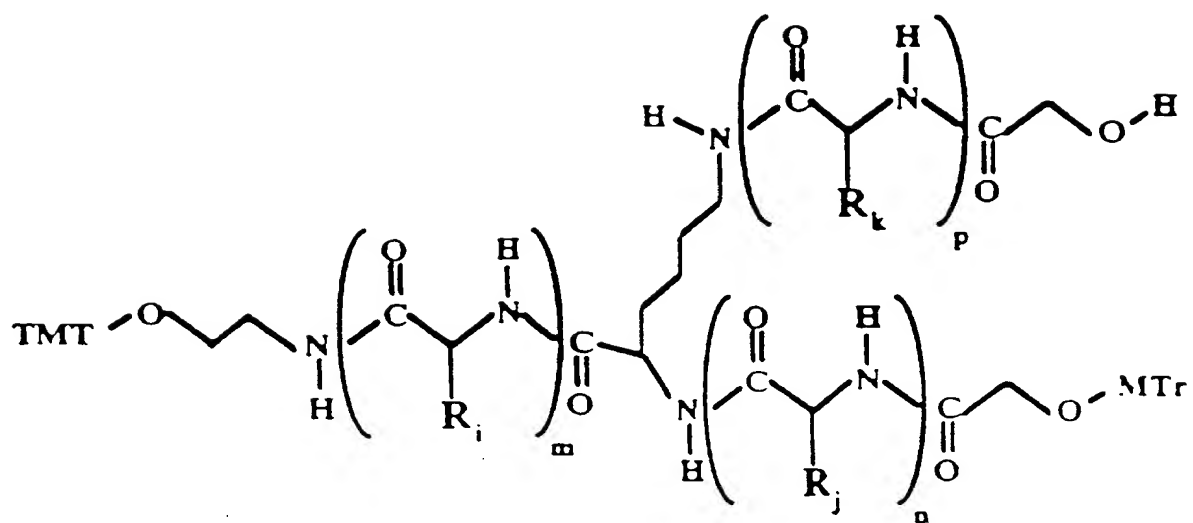


Fig. 11

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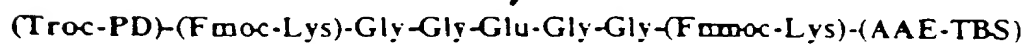
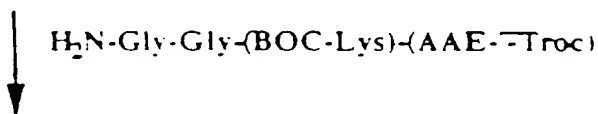
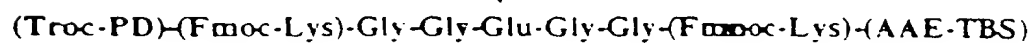
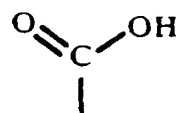
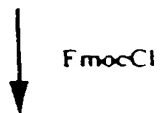
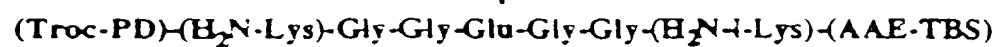
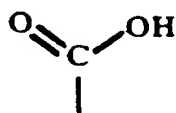
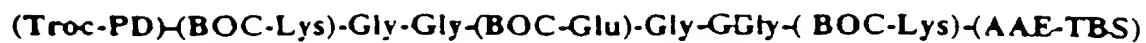


Fig. 13

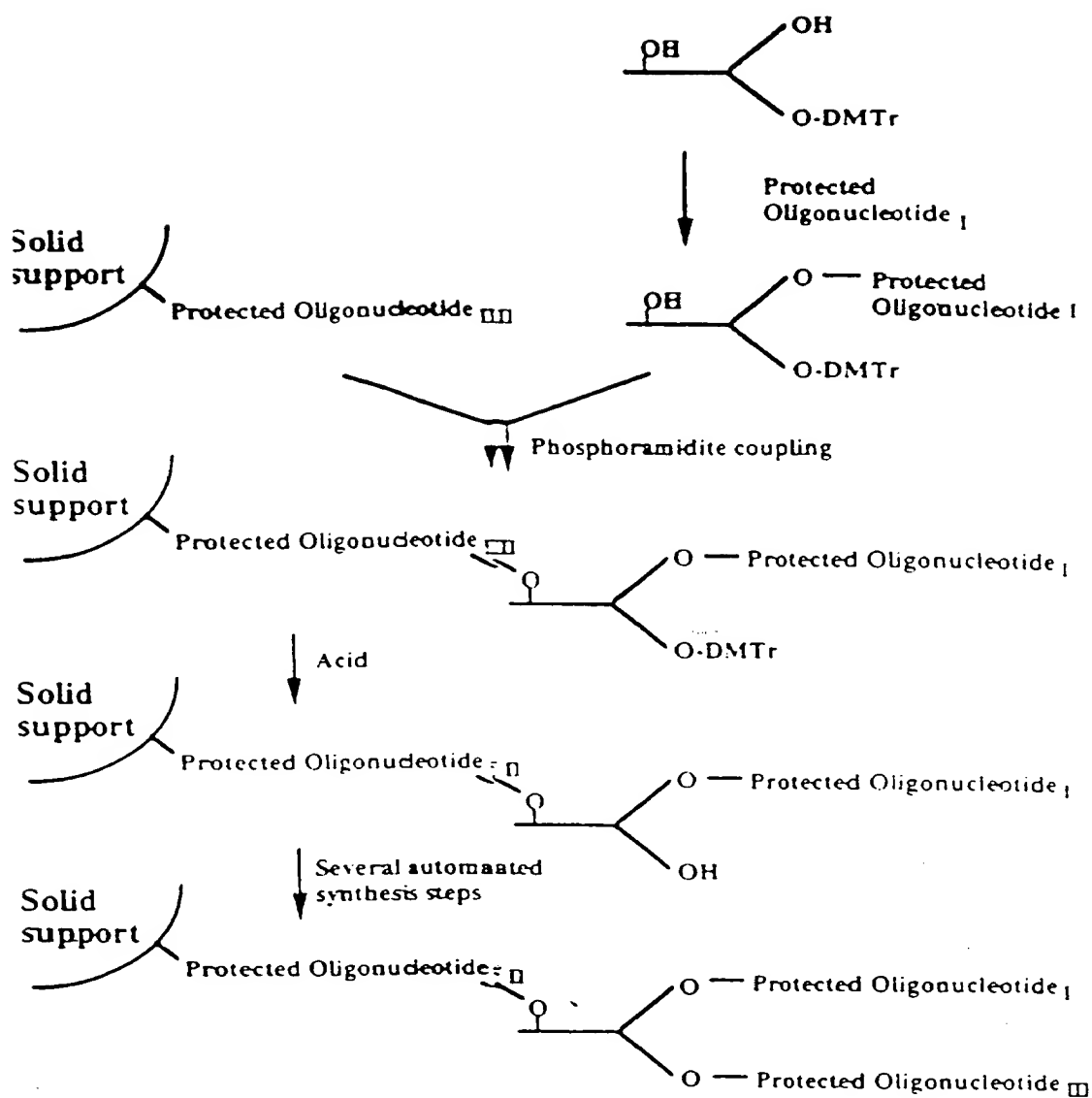
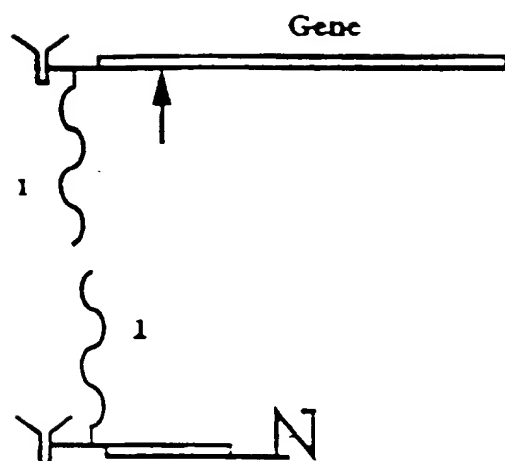


Fig. 14



Antibody



Restriction enzyme



Double helix oligonucleotide



Single stranded oligonucleotide

Fig. 15A

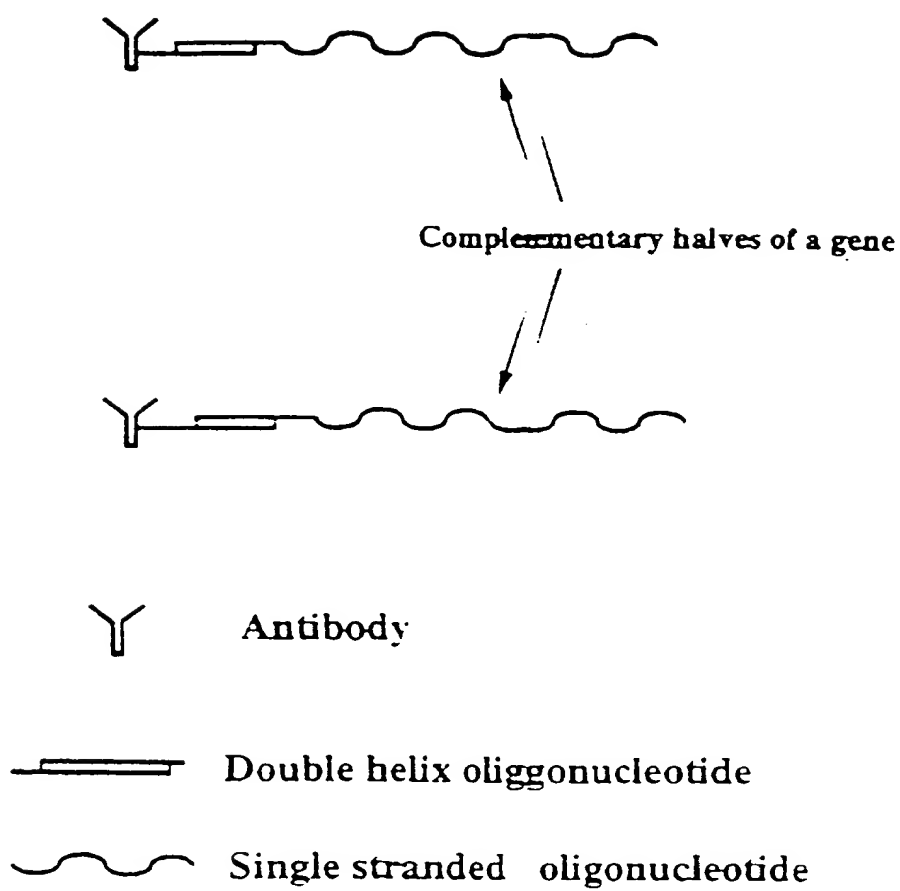


Fig. 15B

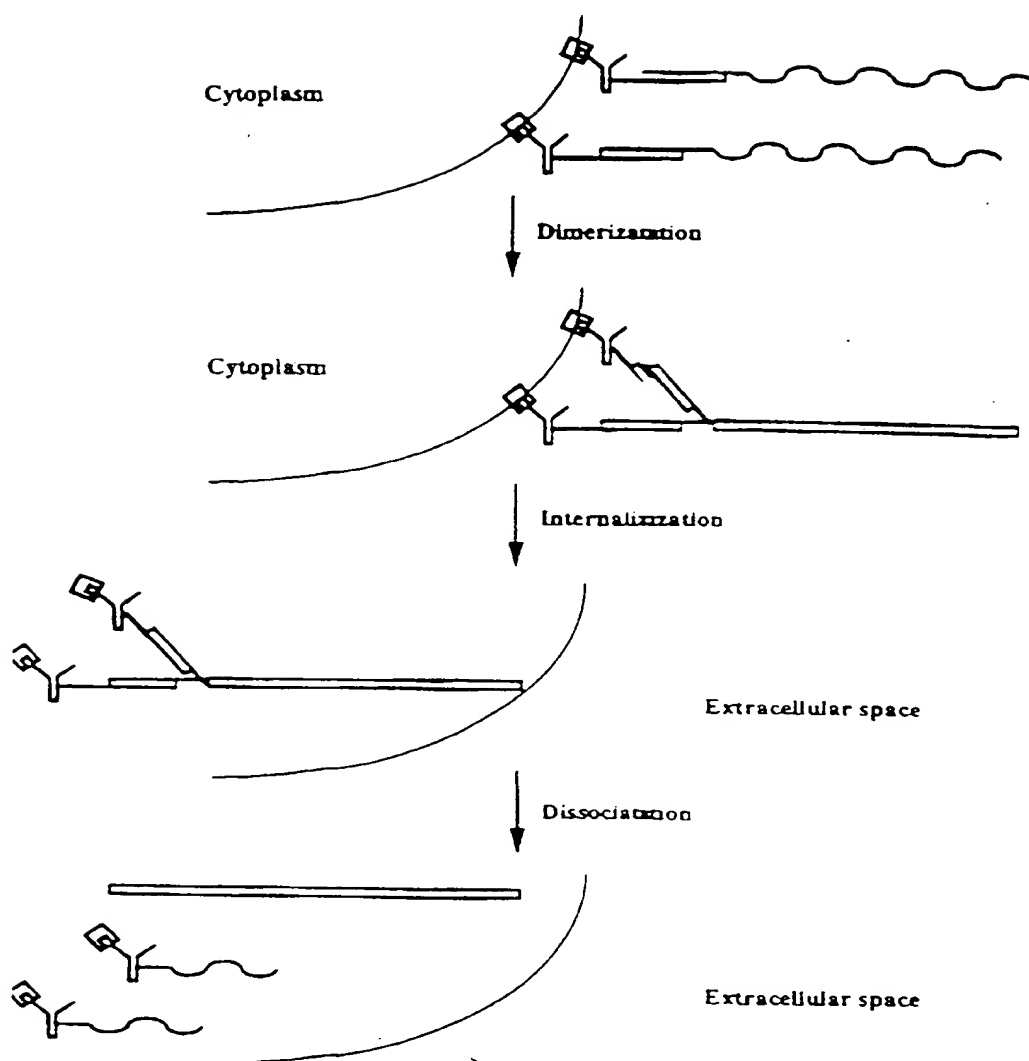


Fig. 15C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13990

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 16/00, 17/00, 17/14

US CL : 530/391.1, 391.5, 391.9, 813

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/391.1, 391.5, 391.9, 813

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS/DIALOG (EMBASE, BIOSYS, LIFESCI, MEDLINE, WPI)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,316,906 (HAUGLAND et al.) 31 May 1994, see entire document.	1-15
Y	US, A, 5,328,985 (SANO et al.) 12 July 1994, see entire document, especially columns 19-20.	1-15

☐

Further documents are listed in the continuation of Box C

☐

See patent family annex.

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